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14. ABSTRACT

African-American men have a higher incidence of prostate cancer and develop prostate cancer at a younger age than white men. Residential segregation may play a role in these observed disparities by reducing African American men's' ability to avoid harmful environmental exposures. Housing is one intermediate factor affected by residential segregation and housing quality and tenure is known to differ between African-Americans and whites. This study will use assess the relationship of both area- and individual-level housing characteristics and prostate cancer risk, age at diagnosis, and disease aggressiveness in African American and white men.

15. SUBJECT TERMS
Prostate cancer, African-American, housing, residential segregation

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I. Introduction

We hypothesize, that race-based residential segregation leads to disparities in both area (census tract/block group) and individual physical and social housing conditions that dispose African-American men to differential environmental conditions that lead to excesses in biological damage, increasing risk for prostate cancer, earlier age of prostate cancer onset, and worse prostate cancer outcomes compared to white men.

Specifically we aim:
1. to determine whether selected *area housing and individual housing status (homeownership, housing density, and other housing factors such as age of structure and heating sources)* are associated with prostate cancer risk, age at diagnosis, and tumor aggressiveness and whether housing status is associated with observed racial differences in these prostate cancer outcomes.

2. to determine, through the use of factor analysis, whether *area housing and individual housing status*, is associated with prostate cancer risk, age at diagnosis, and tumor aggressiveness, through "latent factors" that include diet, physical activity, and genetic polymorphisms and whether those "latent factors" differ by race.

3. to <u>begin</u> to test biological pathways through which *housing status* may impact prostate health outcomes; specifically, whether *housing status* is associated with markers of DNA damage (polycyclic aromatic hydrocarbons DNA-adducts (PAH)) and DNA stability (telomere content) in prostate tumor tissue and tumor-adjacent normal tissue of African-American and white cases.

II. Body

<u>Doctoral Training Program</u>

Between March 2007 and 2008, progress has been made on several components of this pre-doctoral training award and on the research proposed. In addition to meeting with mentors, Dr. Benjamin Rybicki and Dr. Janet Hankin, regularly, I participate in and regularly attend the Multi-Disciplenary Prostate Cancer Working Group meeting organized by Wayne State University, Karmanos Cancer Institute and Henry Ford Health System. The Prostate Cancer Working Group's mission is to support the development of grant proposals directly aimed at racial disparities in prostate cancer and in particular to support, multi-disciplinary projects across the three institutions. In addition, I attend and present my ongoing research at weekly meetings of the HFHS Urology Research Department. This research group includes basic researchers, population scientists and clinician researchers in several areas of genitourinary research including prostate cancer. HFHS has recently established a Disparities Research Collaberative, headed by Christine Joseph, PhD, to organize disparities research within the institution and to aid the development of research methods specifically related to disparities research. I have been asked to serve on the planning committee for the Collaberative, giving me access to several experts in disparities research outside of HFHS as well as inside the institution. In addition, I attended and presented posters at two American Association for Cancer Research conferences, The Science of Cancer Health Disparities (November 2007) and Telomeres and Telomerase in Cancer (December 2007).

Task 1. to determine whether selected *area housing and individual housing status (homeownership, housing density, and other housing factors such as age of structure and heating sources)* are associated with prostate cancer risk, age at diagnosis, and tumor aggressiveness and whether housing status is associated with observed racial differences in these prostate cancer outcomes.

a. Complete the download of census housing data.

b. Complete the capture of individual level housing variables.

c. For assessment of prostate cancer risk, cases and controls will be used in analysis. Age of diagnosis and tumor aggressiveness analyses will include cases only. Hierarchical logistic regression will be performed to assess the relationship of area-level and individual level housing status to prostate cancer risk and tumor aggressiveness (Gleason >7 or pathological stage ≥ T2C).

d. Kaplan Meier survival statistics and Cox proportional hazards models will be used. We anticipate multi-colinearity among housing variables and among other socioeconomic variables such as median household income.

e. Race stratified analyses will also be conducted.

Tasks 1a and 1b have been completed. Task 1b the collection of individual level homeownership data took longer than expected. My department covered the cost of collecting the individual level homeownership data. The price of collection increased from $7 dollars to $15 dollars per subject and had to be spread across twelve months rather than six months time for departmental budget purposes. Individual level homeownership data for 881 subjects is now available for analysis. In addition, we were able to capture housing value. However, because of the increased price of reports age of structure and heating source at the individual level are unavailable for analysis. Those variables are available at the area level, however, and will be incorporated into analyses.

In addition, in the first year of the grant I was assigned a statistical advisor who reviewed the age at diagnosis outcome. After assessment of the case-control nature of the study, Dr. Shultz, senior biostatistician recommended a cohort design as more appropriate for estimating factors that effect age at diagnosis. I am working to establish a prostate cohort from existing administrative data at HFHS that will better serve this outcome.

Race stratified analyses will be conducted once overall analyses are completed.

Task 2. To determine, through the use of factor analysis, whether *area housing and individual housing status,* is associated with prostate cancer risk, age at diagnosis, and tumor aggressiveness, through "latent factors" that include diet, physical activity, and genetic polymorphisms and whether those "latent factors" differ by race.

a. We will conduct factor analysis and we will include measures of housing status individual and area level, diet (total calories, total fat calories, total carbohydrates, selenium, and lycopene), minutes of leisure activity and work, gene polymorphisms.

b. Separate analyses will be conducted by race as well, since component characteristics may be different by race.

In the first year of the study, I was assigned a doctoral level statistics mentor who reviewed the study aims with me. Dr. Lonni Schultz, Senior Biostatistician, raised concerns regarding the high correlation levels within categories of variables, for example high correlation between total

calories and total fat. These correlations were much stronger than correlations across categories of variables for example total fat and gene polymorphisms. Dr. Schultz recommended beginning with a multivariate analyses and potentially multi-level analysis. A diagram of the analysis that is underway is presented in Appendix 1. Shaded boxes indicate data that has been analyzed at the univariate and bivariate levels thus far.

Task 3. To <u>begin</u> to test biological pathways through which *housing status* may impact prostate health outcomes; specifically, whether *housing status* is associated with markers of DNA damage (polycyclic aromatic hydrocarbons DNA-adducts (PAH)) and DNA stability (telomere content) in prostate tumor tissue and tumor-adjacent normal tissue of African-American and white cases.

a. We will use correlations for continuous housing measures and chi square test for categorical housing measures.

b. Race stratified analyses will be conducted.

Now that individual homeownership data is available this task will be completed. In preparation for these analyses, I have assessed whether there are differences in prostate PAH adduct levels based on city of residence (Detroit vs. Non-Detroit), as Detroit has more industry and expressway traffic than surrounding suburban communities. Industry and automobile combustion are known sources of PAH. I found no difference in mean prostate PAH adduct levels between men residing in the city of Detroit and men living in the surrounding suburbs [mean ± sd: Detroit residents' prostate tumor tissue adduct levels .151 ± .05 vs. Non-Detroit .147 ± .06, p=.49, Detroit tumor-adjacent normal prostate tissue adduct levels .249 ± .08 vs. Non-Detroit .244 ± .08, p=.54].

Task 4. Reporting of results

a. Results will be presented at professional meetings

b. At least one manuscript will be submitted for publication

c. Dissertation will be completed.

This task will be completed between December 2008 and the close of the grant in the spring of 2009. Since the Department of Defense prostate meeting will not take place before the end of this project period, I will submit a request in the Fall 2008 to re-budget monies originally planned for travel to the DoD prostate meeting for travel to the AACR Disparities meeting in February 2009 and will plan to present study results at that time.

III. Key Research Accomplishments

- Census data for all 881 study subjects has been captured
- Individual homeownership data on 881 subjects has been captured
- Developed more detailed diagram to accomplish Task 2
- Cleaned data set
- Completed univariate and most bivariate analyses (see Appendix 1 shaded boxes)
- Submitted Transitioning Investigator disparities proposal to the Department of Defense Prostate Research Program for consideration

Please note the manuscripts and posters listed below as research accomplishments are only indirectly related to the aims of this study as they use the same data set. These accomplishments are reported here to show that the training environment is supportive of my career development in prostate cancer and disparities research. See Appendix 2 for copies of manuscripts and Appendix 3 for copies of abstracts.

Manuscripts Published as Part of the Doctoral Training Program

Tang D, Liu JJ, Rundle A, **Neslund-Dudas C**, Savera AT, Bock CH, Nock NL, Yang JJ, Rybicki BA. Grilled meat consumption and PhIP-DNA adducts in prostate carcinogenesis. *Cancer Epidemiol Biomarkers Prev*. 2007 Apr;16(4):803-8.

Nock NL, Tang D, Rundle A, **Neslund-Dudas C**, Savera AT, Bock CH, Monaghan KG, Koprowski A, Mitrache N, Yang JJ, Rybicki BA. Associations between smoking, polymorphisms in polycyclic aromatic hydrocarbon (PAH) metabolism and conjugation genes and PAH-DNA adducts in prostate tumors differ by race. *Cancer Epidemiol Biomarkers Prev*. 2007 Jun;16(6):1236-45.

Tang D, Liu JJ, Bock CH, **Neslund-Dudas C**, Rundle A, Savera AT, Yang JJ, Nock NL, Rybicki BA. Racial differences in clinical and pathological associations with PhIP-DNA adducts in prostate. *Int J Cancer*. 2007 Sep 15;121(6):1319-24.

Neslund-Dudas C, Bock CH, Monaghan K, Nock NL, Yang JJ, Rundle A, Tang D, Rybicki BA. SRD5A2 and HSD3B2 polymorphisms are associated with prostate cancer risk and aggressiveness. *Prostate*. 2007 Nov 1;67(15):1654-63.

Rybicki BA, **Neslund-Dudas C**, Bock CH, Rundle A, Savera AT, Yang JJ, Nock NL, Tang D. Polycyclic Aromatic Hydrocarbon-DNA Adducts in Prostate and Biochemical Recurrence after Prostatectomy.*Clin Cancer Res*. 2008 Feb 1;14(3):750-7.

Abstracts Presented

Henry Ford Health System Research Symposium, Detroit, MI, Apr 13, 2007
A pilot study of telomere repeat binding factor 1 (TRF1) protein expression in prostate tumor and adjacent non-tumor cells of African-Americans and Caucasians.
C. Neslund-Dudas, S.P. Dudas, A.K. Meeker, X. Zhang, A.T. Savera, B.A. Rybicki

AACR - The Science of Cancer Health Disparities, Atlanta, GA, Nov 27-30, 2007
Coffee, beer, and wine consumption and PhIP-DNA adducts in black and white men with prostate cancer. **C. Neslund-Dudas**, D. Tang, C.H. Bock, A. Rundle, N. Nock, J. Beebe-Dimmer, B.A. Rybicki

AACR – Telomeres and Telomerase in Cancer, San Francisco, CA, Dec 6-9, 2007
A pilot study of telomere repeat binding factor 1 (TRF1) and telomere content in prostatectomy specimens of black and white men with prostate cancer. **C. Neslund-Dudas,** S.P. Dudas, A.K. Meeker, X. Zhang, A.T. Savera, R. Mikita, B.A. Rybicki.

IV. Reportable Outcomes

Reportable outcomes are expected for the next review. I anticipate analyses to be complete in November-December 2008 now that data cleaning and acquisition of individual level home ownership is complete. Manuscripts will be prepared between December and the close of the grant period.

V. Conclusion

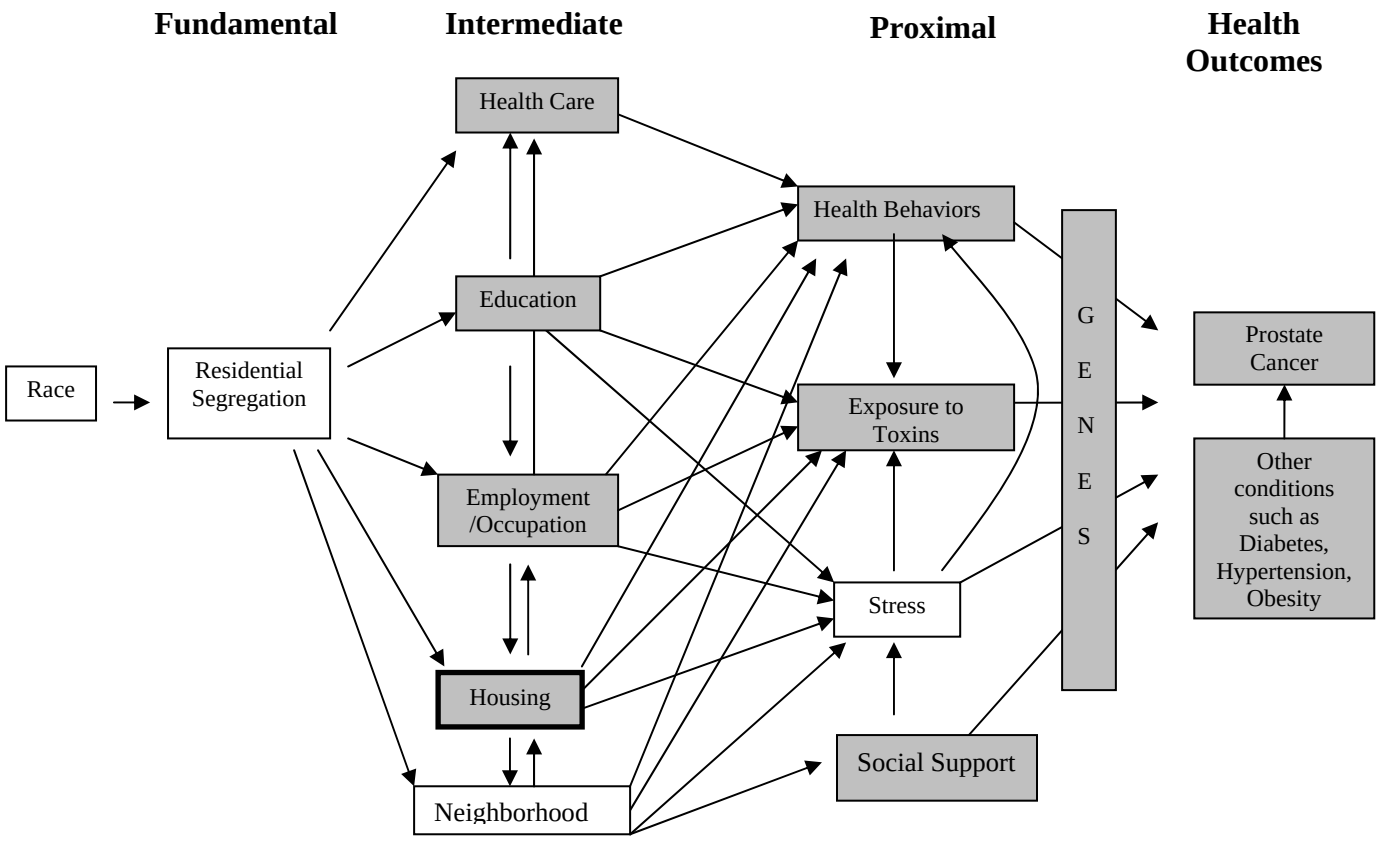
In 2008-2009, the final year of this two year pre-doctoral training grant, I plan to complete the area and individual housing analyses planned in Aims 1 thru 3. I will complete my dissertation and submit manuscripts related to this project. Data acquisition and analysis has moved more slowly than planned. I have worked with my dissertation committee to outline a plan for timely completion of this project which includes a dissertation defense before the end of 2008 and work on manuscripts in the first quarter of 2009, meeting the timeline and goals for this study.

VI. References

None

APPENDIX I

Residential Segregation as a Fundamental Social Cause of Disparities in Prostate Cancer



Note: Shading indicates variables that have been cleaned and assessed on the univariate and bivariate level. Stress will not be included but is listed here as a factor that in the future should be taken into account. Neighborhood variables (for example: median household income, % black residents, % male headed households, % below poverty) are in the process of being assessed at the univariate and bivariate levels.

APPENDIX II

Publications 2007-2008

Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2007 April ; 16(4): 803–808.

Grilled Meat Consumption and PhIP-DNA Adducts in Prostate Carcinogenesis

Deliang Tang[1], **Jason J. Liu**[1], **Andrew Rundle**[2], **Christine Neslund-Dudas**[4], **Adnan T. Savera**[5], **Cathryn H. Bock**[3], **Nora L. Nock**[6], **James J. Yang**[4], and **Benjamin A. Rybicki**[4]

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Abstract

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the major heterocyclic amine generated from cooking meats at high temperatures, and dietary exposures have been shown to induce prostate cancer in rats. PhIP derives its carcinogenic potential through the formation of PhIP-DNA adducts. The purpose of this study was to examine whether self-reported consumption and preparation doneness of grilled meats were associated with PhIP-DNA adduct levels in prostate epithelial cells. The study population consisted of 268 African-American and Caucasian men who underwent radical prostatectomy for prostate cancer. PhIP-DNA adducts in tumor and adjacent nontumor cells were measured using immunohistochemical methods, and dietary meat intake information was based on food frequency questionnaires. Data were analyzed using multivariate linear regression models. After adjusting for age at prostatectomy and race, grilled meat consumption ($P = 0.002$) was significantly associated with higher adduct levels in tumor cells, but this association seemed to be primarily due to consumption of grilled red meats ($P = 0.001$) as opposed to grilled white meat consumption ($P = 0.15$). Among the specific food items, grilled hamburger consumption had the most significant association with adduct level in tumor cells ($P = 0.002$). Similar trends in positive associations with grilled meat consumption and adduct levels were observed in nontumor cells, but none of these associations reached statistical significance. Our results suggest that dietary interventions targeted at lower consumption of grilled red meats may reduce prostate cancer risk via the PhIP prostate carcinogenic pathway.

Introduction

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the most abundant heterocyclic amine (HCA) formed during the cooking of meat (1) and is a potential dietary risk factor for prostate and other cancers. In rats, PhIP preferentially targets the colon and prostate in males, the mammary glands in females, and lymphoid cells in both males and females (2), whereas in mice, it induces lymphoma (3). Subsequent studies have firmly established that PhIP is a

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potent prostate carcinogen in rats (4,5). In humans, meat consumption assessed by food frequency questionnaires has been used as a possible surrogate for PhIP and other HCA intake. Studies have found that intake of grilled meat increased the risk of colorectal adenomas (6) and stomach cancer (7), intake of fried meat increased lung cancer risk (8), and higher estimated HCA intake increased breast cancer risk (9). A large prospective study of men enrolled in the Prostate, Lung, Colorectal, and Ovarian Screening Trial found that the highest quintile of dietary PhIP intake was associated with a 1.2-fold increased risk of prostate cancer (10). Overall, epidemiologic evidence for consumption of meat as a risk factor for prostate cancer risk is equivocal (11). In two of the more recent studies of prostate cancer and meat consumption conducted in the United States, increased meat consumption was positively associated with prostate cancer risk in African Americans, but not Caucasians (12,13). In the United States, African-American men have a 60% higher incidence of prostate cancer compared with whites (14). Coincidentally, mean dietary HCA intake is ~2- to 3-fold greater in African-American males than their white male counterparts (15).

Compared with the use of food frequency questionnaires to estimate HCA intake or urinary excretion to assess metabolism, PhIP-DNA adducts serve as a biomarker of a chemical-specific measure of individual biologically effective dose. PhIP is a promutagen that is efficiently metabolized into reactive species that are direct acting mutagens. Bioactivation of PhIP to carcinogenic species *in vivo* is initiated by N-oxidation of the compound, which is catalyzed by cytochrome P4501A2 (CYP1A2; ref. 16). Subsequent acetylation or sulfation of the *N*-hydroxy-PhIP catalyzed by *N*-acetyltransferases or sulfotransferases generate *N*-acetoxy- or *N*-sulfonyloxy-PhIP, electrophilic compounds that bind covalently to DNA to form PhIP adducts (17,18). The formation of PhIP-DNA adducts via nitrenium ion chemistry results in structural changes in the DNA and possibly mutations in genes controlling cell proliferation, thus leading to tumor formation (19). Human prostate cells metabolize PhIP to its mutagenic form (20,21) and form PhIP-DNA adducts after being exposed to PhIP *in vivo* (22-24).

The content of PhIP in cooked meats varies by both the type of meat and its method of preparation (25,26), but the assessment of individual human exposure is very complex with estimated PhIP exposure levels varying by at least two orders of magnitude (27). Recent studies that link survey data to food databases of HCA content have estimated that pan frying and chicken are the cooking method and meat that comprise the primary source of dietary PhIP exposure in American men (15,28), but these studies rely on the linkage of two data sources with a large amount of variation. In addition, most studies have consistently found that grilling/barbecuing is the cooking method that generally produces the highest HCA content (25,28, 29). In any particular population, the relationship between dietary consumption of PhIP and its biologically effective dose will depend on local dietary habits and cooking methods, individual susceptibilities in PhIP metabolism, and the target organ of interest.

If PhIP is an important prostate cancer risk factor, then identification of dietary sources of PhIP correlated with a marker of its biologically effective dose, PhIP-DNA adducts, in the prostate will provide useful data for future dietary chemoprevention. In the present study, we tested for associations between PhIP-DNA adduct level in prostatic epithelial cells and known dietary sources of PhIP exposure, namely, consumption of grilled and overcooked meats.

Materials and Methods

Study Sample

The study population consisted of men who were part of the Henry Ford Health System (HFHS). The HFHS is composed of an 800-bed hospital in the city of Detroit, 3 smaller hospitals in surrounding suburbs, and 31 medical clinics located throughout the metropolitan Detroit area. Eligible cases used the HFHS as their primary source of health care, lived in the

study area at time of recruitment, had no other serious medical problems that would preclude participation, and had no previous history of prostate cancer. Potential cases were identified by HFHS pathology reports of primary adenocarcinoma of the prostate. Cases recruited for study were sent a letter introducing the study protocol, followed by a phone call from a study interviewer. Those who agreed to participate were asked to complete a two-part interviewer-administered risk factor questionnaire (the first part was conducted over the phone, and the second part was done in person) and donate a blood sample for DNA analysis. Race was self-reported by participants. All study protocols were approved by the Henry Ford Hospital Institutional Review Board.

Between July 1, 2001 and December 31, 2004, we attempted to enroll 863 men who had been diagnosed with prostate cancer within the last 2 years as part of a prostate cancer case-control study, and 668 agreed to participate (77%). During the course of enrollment, 8 cases were found ineligible, and 23 cases did not complete the study protocol, resulting in final study participation percentages of 74% (637/855). Of these 637 cases, 434 (68%) underwent radical prostatectomy. Cases undergoing prostatectomy were, on average, younger (61.0 years versus 65.1 years, $P < 0.001$) but did not differ by race (43.2% African American in both groups) or by Gleason score (biopsy Gleason ≥ 7: 43.1% versus 42.1%). The present study includes the first 268 prostatectomy patients who had tissue samples available for immunohistochemical studies of PhIP-DNA adduct determination. The demographic and clinical characteristics of the study population are shown in Table 1. Date of surgery and tumor grade were abstracted from the surgical pathology report.

Food Questionnaires

Dietary intake as well as food preparation method and doneness were ascertained using questions adapted from a validated questionnaire (30). Grilled meats were defined as meats cooked over charcoal or a hot gas flame. Meat servings and preparation doneness data were collected through the following questions. For determining grilled meat servings, the question of “in the summer months, did you eat meats cooked on an outdoor grill or barbecue” was asked. If the respondent answered yes for outdoor grilled meat intake, then the following questions of “in the summer months, how often did you eat the following grilled meats (steak or pork chops, hamburgers, hot dogs, chicken with skin, chicken without skin, fish)” were asked. For determining smoked meat servings, the questions of “how often did you eat smoked ham, turkey, or other smoked meats” and “how often did you eat smoked fish” were asked. Preparation doneness was determined for grilled meats in grilled steaks or chops, grilled hamburgers, and grilled hot dogs through the questions of “when you ate grilled (steak or pork chops, hamburgers, and hot dogs), how were they cooked?” The number of servings categories include “<1 per month,” “1 to 4 times per month,” “5 to 9 times per month,” “10 to 15 times per month,” and “>15 times per month.” The preparation doneness categories include “rare,” “medium,” “well done,” and “very well done.”

Pathology

H&E-stained slides of study cases were reviewed by the study pathologist (A.T. Savera) to confirm the diagnosis and to identify a paraffin block with sufficient tumor and nontumor prostatic tissue staining. For each patient sample, a microtome was used to cut five consecutive sections (5 μmol/L thick) from the tissue block. One slide was H&E stained and examined by the study pathologist who circled two separate areas of tumor and normal cell populations to be used for adduct scoring.

Immunohistochemistry

Immunohistochemical studies were done as described by Takahashi et al. (31) and Zhu et al. (32). Sensitivity and specificity of the antibody were described previously (31). The sensitivity

was one to two adducts per 10^7 nucleotides, whereas the target of the anti–PhIP-DNA adduct antibody was PhIP-bound DNA rather than unbound PhIP or its metabolite (31). The paraffin-embedded sections were baked at 59°C for 1 h, deparaffinized in xylene, and rehydrated in serial alcohol. Endogenous peroxidase activity was blocked using 0.3% H_2O_2 in methanol for 20 min. After treatment using RNase and proteinase K, the sections were blocked using 3% bovine serum albumin and normal goat serum. The primary anti–PhIP-DNA adduct polyclonal antibody was provided by Dr. Shirai (Nagoya City University Medical School, Nagoya, Japan). The polyclonal antibody was incubated with the sections at 4°C overnight in a humid chamber at a dilution of 1:750. In addition, the biotinylated secondary antibody was incubated with the sections at room temperature for 30 min at a dilution of 1:200. The antibody complex was detected using an avidin-biotin-peroxidase complex solution and visualized using 3,3′-diaminobenzidine (Zymed Laboratories, Inc., San Francisco, CA). A negative control was included in each experiment by omitting the primary antibody. The staining specificity was confirmed using the primary antibody that had been pre-absorbed with 2 or 20 μg/mL DNA extract from MCF-7 cells treated with 150 μmol/L *N*-hydroxy-PhIP. A cytospin sample of MCF-7 cells without PhIP treatment was included in each batch of staining. Staining was measured by absorbance image analysis using a Cell Analysis System 200 microscope as described previously (33). Absorbance of light at a wavelength of 500 nmol/L was measured because methyl green does not absorb light at this wavelength, whereas diaminobenzidine does. For each prostate specimen, two technicians independently scored 50 epithelial cells (five fields with 10 cells per field scored) in the two areas (tumor and nontumor) circumscribed by the study pathologist. The final score was the mean of the two technicians' scores. Scored cells were selected to be representative, in terms of intensity, of the cells in the field. Staining intensity was represented by the absorbance value.

Statistical Analyses

Multivariate linear regression analyses were used to determine whether meat consumption was associated with PhIP-DNA adduct levels in nontumor and tumor prostatic epithelial cells. Potential batch effects in the PhIP-DNA adduct assay were taken into account by assaying a control slide with each experimental batch to compute a batch correction factor that was the difference between the adduct level of the control slide in a single batch and the mean adduct level of the control slides across all batches. The batch-adjusted adduct level was the crude adduct level minus the batch correction factor. This approach was used in our previous studies involving PAH-DNA (33) and PhIP-DNA[7] adducts. The distribution of adjusted adduct levels was found to be close to normal, and hence, no log transformation was necessary for that variable. Due to the low number of subjects who reported eating many servings of individual meats, as well as rare and very well-done meat, both number of servings and preparation doneness variables were dichotomized for multiple regression analyses. For variables of consumption, study subjects were grouped into consumers or nonconsumers. For variables of preparation doneness, subjects were grouped into "rare and medium" meat consumers or "well done and very well done" meat consumers. For the combined meat consumption variables, individual dichotomous meat consumption variables were scored as a "1" for those who consumed each meat and "0" for those who did not and summed across all meat categories. Total grilled white meat consumption included intake of grilled chicken with and without skin and fish. Total grilled red meat consumption included intake of grilled hamburger, hot dog, and steak/pork chop.

To determine whether our findings were specific to red meat consumption prepared on the grill during the summer, we also examined whether PhIP-DNA adduct level was associated with

[7]D. Tang, C.H. Bock, C. Neslund-Dudas, et al. Race-Specific Determinants of PhIP-DNA Adducts in Prostate Cancer. Carcinogenesis, submitted 2006.

season. Date of prostatectomy was grouped into one of the four different seasons, and an ANOVA was done to test for heterogeneity of mean adduct levels across the four groups.

All models adjusted for age at prostatectomy and race (African American or Caucasian). Associations between meat consumption variables and PhIP-DNA adduct levels were examined separately for nontumor and tumor prostatic epithelial cells.

Results

PhIP-DNA adduct level was found to be significantly higher in nontumor cells (mean absorbance, 0.17) compared with that in tumor cells (mean absorbance, 0.10; $P < 0.0001$). Race and age at prostatectomy were not significantly associated with adduct level in either nontumor or tumor cells. Race was significantly associated with steak consumption, hamburger consumption, chicken with skin consumption, and chicken without skin consumption.

PhIP-DNA adduct levels across different meat consumption categories are shown in Table 2. With the exception of grilled fish, those who consumed grilled meats had higher mean PhIP-DNA adduct levels in both nontumor and tumor cells than those who did not. Those who consumed three different specific red meats had a mean nontumor cell adduct level of 0.177 ± 0.038 absorbance, compared with 0.163 ± 0.048 absorbance ($P = 0.057$) for those who consumed no red meat at all, whereas those who consumed three different specific red meats have a mean tumor cell adduct level of 0.113 ± 0.025 absorbance, compared with 0.0988 ± 0.025 absorbance for those who consumed no red meat at all ($P = 0.001$). In contrast, higher PhIP-DNA adduct levels were not observed across the four levels of doneness for the three food groups for which this meat preparation question was asked (Table 3).

After adjusting for age at prostatectomy and race, total meat consumption ($\beta = 0.002$, $P = 0.002$) and total grilled red meat consumption ($\beta = 0.005$, $P = 0.001$) were found to be significantly associated with adduct level in tumor cells (Table 4). Total grilled white meat consumption was not significantly associated with adduct level in either tissue type.

In analyses involving specific grilled red meats (Table 4), the association between grilled hamburger consumption and PhIP-DNA adduct level was marginally significant in nontumor cells ($\beta = 0.010$, $P = 0.077$), but was significant in tumor cells ($\beta = 0.011$, $P = 0.002$). Grilled steak/pork chop ($\beta = 0.008$, $P = 0.020$) and grilled hot dog consumption ($\beta = 0.009$, $P = 0.009$) were also significantly associated with adduct level in tumor cells. Other specific meat items in which consumption was associated with increased adduct level include grilled chicken with skin consumption ($\beta = 0.008$, $P = 0.019$) in tumor cells.

Next, we ran a series of multivariate models that included covariates for all six of the specific meat consumption categories to adjust for interdependence among the six categories. Because no significant associations were found between the different food doneness categories and adduct level, we chose not to include any variables for doneness in our multivariate models. First, we ran two saturated models for adduct levels of nontumor and tumor cells, forcing all six meat category variables into the model as well as covariates adjusting for race and age at prostatectomy. In general, effect estimates for all food variables tended to decrease both in magnitude and statistical significance in the saturated models, with none of the specific grilled red meat consumption categories significantly associated with adduct level in either nontumor or tumor cells (Table 5). We then used backward elimination regression to obtain best-fitting models of specific meat consumption categories for adduct levels after adjusting for age at prostatectomy and race. For nontumor cells, only grilled hot dog consumption was retained in the model ($\beta = 0.010$, $P = 0.076$), whereas for tumor cells, only grilled hamburger consumption was retained ($\beta = 0.011$, $P = 0.002$).

Our analysis comparing PhIP-DNA adduct levels by the season in which the prostatectomy was done showed that there was no significant seasonal variations for either nontumor or tumor cells.

Discussion

Our study results are novel in linking dietary PhIP exposure with a biologically effective dose biomarker, PhIP-DNA adducts, in the prostate. Although we could not examine whether higher PhIP-DNA adduct levels increased prostate cancer risk, a recent prospective human study found that the highest quintile of dietary PhIP intake was associated with a 1.2-fold increased risk of prostate cancer (10). Although several previous studies have examined biomarkers of PhIP exposure such as urine metabolites (34-36) and hair (37), only a few studies have attempted to correlate a biologically effective measure of PhIP exposure, PhIP-DNA adduct levels, in humans with self-reported exposure histories (32,38). Only a few studies have assayed for PhIP-DNA adducts in the tissues taken from the target organ in which the cancer occurred (32,39). In a breast cancer study, no direct correlation between different types of meat consumption and PhIP-DNA adducts levels was found, but a suggestive interaction between *N*-acetyltransferase genotype and well-done meat consumption was reported (32). In a pancreatic cancer study, PhIP-DNA adducts were detected in human pancreatic tissue samples obtained from patients with unknown exposure to HCA (39). In the present study, we examined self-reported grilled meat consumption as a potential dietary source of PhIP exposure and found that grilled red meat and total meat consumption were significantly associated with adduct levels in prostatic tumor cells.

In general, we found the strongest associations between specific types and amount of grilled meat consumption and PhIP-DNA adduct level in tumor cells. Differences in the cellular microenvironment of tumor and nontumor cells, such as aberrant methylation, may lead to decreased activity of enzymes involved in the detoxification of PhIP in tumor cells, which in turn could result in a stronger correlation between reported eating habits and a tissue-based biomarker of PhIP in tumor cells. For example, silencing of GSTP1 through hyper-methylation has been observed in prostate tumor cells but not normal cells (40), and *in vitro* studies have shown that GSTP1 expression is correlated with PhIP-DNA adduct levels in the prostate (21). In terms of consumption, PhIP-DNA adduct level was linearly associated with total grilled red meat consumption in both nontumor and tumor cells. This finding was consistent with previous animal studies, which found a dose-response relationship between PhIP intake and PhIP-DNA adduct formation (41,42). It was also consistent with epidemiologic studies involving humans, which showed associations between red meat intake and increased risk of colorectal adenomas (43), lung (44), stomach (7), and breast cancers (9,45). Our food questionnaire was limited to only several types of grilled meats, but given our results, it would be interesting to examine whether consumption of other grilled meats, such as grilled bacon or grilled sausage, is associated with PhIP-DNA adduct level in prostate cells. The main limitation of assessing the contributions of the consumption of specific meats on adduct levels using the saturated model is that these consumption categories are highly correlated to each other. In our study, specific red meat items that were significantly associated with PhIP-DNA adduct level in tumor cells when they were analyzed separately lost their significance when put together in the same multivariate model.

We did not find any association between preparation doneness and adduct level. Recall error and exposure misclassification may have contributed to our lack of finding an association between PhIP-DNA adduct level and meat doneness. Because the hamburgers, steaks, and hot dogs in this study were all grilled outdoors, there was a lack of a controlled standard for doneness levels. It was likely that subjects simply ascribed their preference for meat doneness in restaurants to the doneness of outdoor grilled meats, although the restaurant preference may

not reflect the actual doneness level of the outdoor grilled meats according to a fixed standard, because no definitions or pictures of the four doneness levels were presented to the subjects. This notion is supported by a previous doneness exposure indicator study, which found that HCA levels in home-cooked meat samples were significantly different when samples were visually classified for doneness, but not when self-reported doneness preference was used to classify doneness (46). Dietary PhIP intake is thought to be underestimated by food frequency questionnaires primarily because of the difficulty in accurately assessing cooking methods that produce high PhIP levels (47). Furthermore, whereas studies of meat samples show that HCA levels vary significantly by doneness level (15,25,29), the majority of the variation in dietary PhIP intake assessed by food frequency questionnaires is generally accounted for by the type of food and, secondarily, the cooking method (28,48). This likely explains why a previous study of dietary HCA intake and prostate cancer risk found an association between consumption of well-done beefsteak and prostate cancer, but failed to find any overall association between meat doneness and prostate cancer risk (49).

The questionnaire data we collected in the present study did not allow us to examine year-round dietary intake of individual meat items nor all the various methods of food preparation. In fact, the associations we found with PhIP-DNA adduct level in the prostate were specific to meat consumption during the summer months. However, consumption of grilled red meat and hamburger in the summer months could also reflect consumption of these foods at different times of the year and cooked by different methods. Because PhIP-DNA adducts are gradually repaired in cells following their formation (50), we tested whether the season of surgery affected adduct level after controlling for intake level of various meats, but found no significant association between the date of prostate surgery and adduct level. This suggests that grilled red meat consumption did not increase significantly in our sample during the summer months, and that PhIP-DNA adduct level in the prostate is associated more with meat consumption rather than the specific method of cooking.

In summary, we have shown that in men with prostate cancer, consumption of certain types of meat with known high PhIP content is directly related to PhIP-DNA adduct level in tumor and nontumor prostate cells. These results may have important implications with regard to preventive strategies in prostate cancer. Although epidemiologic studies showing a direct link between PhIP-DNA adduct level and increased prostate cancer risk are still lacking, strong evidence exists from animal studies (4,5,51-53) that PhIP is involved in prostate carcinogenesis. Our results suggest that grilled red meat consumption is an important factor to consider in the study of the PhIP prostate carcinogenic pathway in humans.

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Table 1

Characteristics of study population (N = 268)

Characteristic	Mean ± SD
Age at prostatectomy (y)	61.3 ± 6.9
Prostate specific antigen at diagnosis (ng/mL)	7.0 ± 6.3
Race	Number (%)
Caucasian	163 (60.8)
African American	105 (39.2)
Total Gleason grade	
5	1 (0.4)
6	85 (32.7)
7	128 (49.2)
8	29 (11.2)
9	17 (6.5)

Table 2

Mean PhIP-DNA adduct level in prostate nontumor and tumor cells of prostate cancer cases across meat consumption categories (N = 268)

Specific meats	Nontumor					Tumor				
	Consumers		Nonconsumers			Consumers		Nonconsumers		
	n	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*P* value	*n*	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*P* value
Grilled steak/ pork Chop	144	0.171 ± 0.039	124	0.165 ± 0.049	0.28	144	0.108 ± 0.025	124	0.101 ± 0.029	0.036
Grilled hamburger	124	0.173 ± 0.042	144	0.165 ± 0.046	0.14	124	0.109 ± 0.029	144	0.101 ± 0.025	0.01
Grilled hot dog	84	0.175 ± 0.041	184	0.165 ± 0.045	0.076	84	0.111 ± 0.025	184	0.102 ± 0.028	0.008
Grilled chicken with skin	91	0.173 ± 0.039	177	0.166 ± 0.046	0.22	91	0.111 ± 0.026	177	0.102 ± 0.027	0.012
Grilled chicken without skin	99	0.172 ± 0.043	169	0.166 ± 0.045	0.27	99	0.106 ± 0.028	169	0.105 ± 0.027	0.76
Grilled fish	52	0.167 ± 0.042	216	0.169 ± 0.045	0.83	52	0.104 ± 0.024	216	0.105 ± 0.028	0.75

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Table 3

Mean PhIP-DNA adduct level in prostate nontumor and tumor cells of prostate cancer cases across meat preparation categories

Meat category	Doneness level								*P* value
	Rare		Medium		Well done		Very well done		
	n	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	*n*	Absorbance (mean ± SD)	
Nontumor									
Grilled steak/pork chop (*n* = 143)	7	0.161 ± 0.035	73	0.172 ± 0.041	58	0.173 ± 0.039	5	0.157 ± 0.027	0.81
Grilled hamburger (*n* = 124)	3	0.149 ± 0.018	40	0.174 ± 0.039	75	0.173 ± 0.044	6	0.167 ± 0.046	0.78
Grilled hot dog (*n* = 84)	3	0.157 ± 0.0088	12	0.191 ± 0.048	58	0.173 ± 0.039	11	0.177 ± 0.043	0.46
Tumor									
Grilled steak/pork chop (*n* = 143)	7	0.109 ± 0.020	73	0.109 ± 0.025	58	0.109 ± 0.026	5	0.0934 ± 0.024	0.55
Grilled hamburger (*n* = 124)	3	0.0916 ± 0.0095	40	0.113 ± 0.027	75	0.109 ± 0.030	6	0.105 ± 0.037	0.64
Grilled hot dog (*n* = 84)	3	0.104 ± 0.020	12	0.121 ± 0.027	58	0.109 ± 0.024	11	0.114 ± 0.027	0.49

Table 4

Association between meat consumption and PhIP-DNA adduct level, adjusted for age at prostatectomy and race (N = 268)

Food category	Nontumor		Tumor	
	β (95% CI)	*P* value	β (95% CI)	*P* value
Combined meats*				
Grilled red meat	0.004 (-9×10^{-5}, 0.009)	0.055	0.005 (0.002, 0,007)	0.001
Grilled white meat	0.004 (−0.002, 0.009)	0.224	0.003 (−0.001, 0.006)	0.146
All meat†	0.002 (-2×10^{-4}, 0.005)	0.072	0.002 (0.001, 0.004)	0.002
Specific meats‡				
Grilled steak/pork chop	0.007 (−0.004, 0.017)	0.225	0.008 (0.001, 0.014)	0.020
Grilled hamburger	0.010 (−0.001, 0.021)	0.077	0.011 (0.004, 0.018)	0.002
Grilled hot dog	0.010 (−0.001, 0.022)	0.076	0.009 (0.002, 0.016)	0.009
Grilled chicken with skin	0.007 (−0.005, 0.018)	0.241	0.008 (0.001, 0.015)	0.019
Grilled chicken without skin	0.008 (−0.004, 0.019)	0.181	0.002 (−0.005, 0.009)	0.510
Grilled fish	−0.001 (−0.015, 0.012)	0.877	−0.001 (−0.009, 0.008)	0.856

*β estimate represents increment of adduct level increase associated with consumption of each additional meat item in this category.

†Grilled meats and smoked meats.

‡β estimate represents increment of adduct level increase associated with consumption of meat item.

Table 5

Multivariate modeling of consumption of specific meats and PhIP-DNA adduct level, adjusted for age at prostatectomy and race (N = 268)

Food category	Nontumor		Tumor	
	β^* (95% CI)	*P* value	β (95% CI)	*P* value
Grilled steak/pork chop	−0.001 (−0.016, 0.014)	0.876	0.001 (−0.008, 0.010)	0.763
Grilled hamburger	0.005 (−0.011, 0.021)	0.547	0.008 (−0.002, 0.018)	0.102
Grilled hot dog	0.007 (−0.007, 0.022)	0.311	0.004 (−0.004, 0.013)	0.334
Grilled chicken with skin	0.003 (−0.011, 0.017)	0.701	0.004 (−0.005, 0.012)	0.380
Grilled chicken without skin	0.006 (−0.007, 0.019)	0.377	−0.002 (−0.010, 0.006)	0.659
Grilled fish	−0.007 (−0.021, 0.008)	0.378	−0.005 (−0.014, 0.004)	0.276

* β estimate represents increment of adduct level increase associated with consumption of meat item.

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Associations between Smoking, Polymorphisms in Polycyclic Aromatic Hydrocarbon (PAH) Metabolism and Conjugation Genes and PAH-DNA Adducts in Prostate Tumors Differ by Race

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Abstract

Polycyclic aromatic hydrocarbon (PAH)-DNA adducts may induce mutations that contribute to carcinogenesis. We evaluated potential associations between smoking and polymorphisms in PAH metabolism [CYP1A1 Ile^{462}Val, CYP1B1 Ala^{119}Ser and Leu^{432}Val, microsomal epoxide hydrolase (mEH) Tyr^{113}His and His^{139}Arg, CYP3A4 A(−392)G] and conjugation [glutathione *S*-transferase (GST) M1 null deletion, GSTP1 Ile^{105}Val] genes and PAH-DNA adduct levels (measured by immunohistochemistry) in tumor and nontumor prostate cells in 400 prostate cancer cases. Although no statistically significant associations were observed in the total sample, stratification by ethnicity revealed that Caucasian ever smokers compared with nonsmokers had higher adduct levels in tumor cells (mean staining intensity in absorbance units ± SE, 0.1748 ± 0.0052 versus 0.1507 ± 0.0070; $P = 0.006$), and Caucasians carrying two mEH ^{139}Arg compared with two ^{139}His alleles had lower adducts in tumor (0.1320 ± 0.0129 versus 0.1714 ± 0.0059; $P = 0.006$) and nontumor (0.1856 ± 0.0184 versus 0.2291 ± 0.0085; $P = 0.03$) cells. African Americans with two CYP1B1 ^{432}Val compared with two ^{432}Ile alleles had lower adducts in tumor cells (0.1600 ± 0.0060 versus 0.1970 ± 0.0153; $P = 0.03$). After adjusting for smoking status, carrying the putative “high-risk” genotype combination, the faster metabolism of PAH-epoxides to PAH-diol-epoxides (CYP1B1 ^{432}Val/Val and mEH ^{139}Arg/Arg) with lower PAH-diol-epoxide conjugation (GSTP1 ^{105}Ile/Ile), was associated with increased adducts only in Caucasian nontumor cells (0.2363 ± 0.0132 versus 0.1920 ± 0.0157; $P = 0.05$). We present evidence, for the first time in human prostate that the association between smoking and PAH-DNA adducts differs by race and is modified by common genetic variants.

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Introduction

Although prostate cancer is the most commonly diagnosed nonskin cancer and the third leading cause of cancer death among men in the United States (1), increasing age, ethnicity, and family history are the only established risk factors for this disease (2,3). African Americans, in particular, present at an earlier age and with more advanced disease and have higher mortality rates compared with Caucasians (4). Having a strong family history suggests the presence of a highly penetrant gene, but, to date, no single gene which can account for the majority of prostate cancers has been identified. Thus, the pathogenesis of prostate cancer likely involves a complex interplay between multiple low penetrant genetic and environmental factors.

Polycyclic aromatic hydrocarbon (PAH) exposure from cigarette smoke (5-7), grilled meats (8), and various petroleum-related occupations (9-11) may play a role in prostate cancer. Although associations between smoking and these other PAH sources and prostate cancer have been equivocal, PAH require metabolic activation and subsequent binding to DNA (forming bulky “PAH-DNA adducts”) to exert their carcinogenic action (12). Therefore, functional polymorphisms in genes that metabolize PAHs and detoxify their reactive derivatives should be considered when evaluating potential effects of PAH exposure sources. Furthermore, many prior studies have relied upon self-reported measures from a single source; however, PAH-DNA adducts serve as a biological marker of the effective PAH dose from all sources, particularly when quantified in the target tissue. We previously observed that PAH-DNA adducts are present in human prostate cancer cells and vary with tumor characteristics (13).

In terms of PAH metabolism, parent compounds, such as benzo(*a*)pyrene, are initially metabolized by CYP1A1 or CYP1B1 (14,15) to an epoxide [benzo(*a*)pyrene-7,8-epoxide] and subsequently hydrolyzed by microsomal epoxide hydrolase (mEH) to a dihydrodiol [benzo (*a*)pyrene-7,8-dihydrodiol]. CYP1A1, CYP1B1, or CYP3A4 (16) can then transform the dihydrodiol to a highly reactive diol-epoxide [benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide, BPDE] that can covalently bind to DNA, creating a PAH [BPDE]-DNA adduct which may, in turn, induce mutation(s), predominantly in the form of G to T transversions (17). Although mEH (18) and CYP1B1 (19) are expressed in the prostate, CYP1A1 may only be induced under androgen dependency (20) and CYP3A4 may require vitamin D receptor mediation (21). Interestingly, CYP1B1 is highly expressed in the peripheral zone where most prostate cancers arise (22). The CYP1A1 $Ile^{462}Val$ and CYP3A4 A(−392)G polymorphisms have variant alleles with higher enzymatic activity compared with their respective wild-type alleles (23,24), and the activity of the CYP1B1 $Ala^{119}Ser$ and $Leu^{432}Val$ variants is substrate dependent with $^{432}Val/^{119}Ala$, having slightly higher activity in metabolizing benzo(*a*)pyrene-7,8-dihydrodiols but slightly lower activity in metabolizing parent benzo(*a*)pyrene than $^{432}Leu/^{119}Ala$ (15). Effects of the mEH $Try^{113}His$ and mEH $His^{139}Arg$ polymorphisms remain unclear; an earlier study reported that $^{113}Tyr/^{139}Arg$ had the most activity in hydrolyzing benzo(*a*)pyrene-epoxides to benzo(*a*)-pyrene-dihydrodiols (25), but recent work shows the $^{113}Tyr/^{139}His$ combination may be the most active (26). The CYP1A1 $Ile^{462}Val$, CYP1B1 $Leu^{432}Val$, CYP3A4 A(−392)G polymorphisms have been equivocally associated with prostate cancer (27-31), which may be attributed, in part, to heterogeneity in PAH exposure. In prostate cancer, only one (null) finding has been reported for the mEH $His^{139}Arg$ polymorphism (32) and no studies have examined the mEH $Tyr^{113}His$ polymorphism.

Before a PAH-diol-epoxide metabolite can adduct DNA, it may be detoxified by enzymes in the glutathione *S*-transferase (GST) family. In particular, GSTM1 and GSTP1 exhibit substrate specificity for PAH-diol-epoxides (33) and are expressed in the prostate (34-36). Although GSTT1 is highly expressed in the prostate (37), it does not seem to be involved in PAH metabolite conjugation (38). GSTP1 has two polymorphisms: $Ile^{105}Val$ and Ala114Val ($Ile^{105}Val$ is located near the hydrophobic binding site and has more influence on activity; ref.

39). The effect of the GSTP1 Ile^{105}Val polymorphism is substrate dependent with the ^{105}Val allele having a higher affinity for conjugating the most reactive PAH-diol-epoxides (40,41). GSTM1 has a polymorphism that leads to complete loss of protein (GSTM1 null deletion), and this polymorphism, as well as GSTP1 Ile^{105}Val, has been associated with increased prostate cancer risk in some studies (42-44) but decreased risk in others (45-47), which may also be due, in part, to heterogeneity in PAH exposure.

Although associations between a few of the aforementioned polymorphisms in metabolism and conjugation genes and PAH-DNA adduct levels have been examined in human lung (48) and breast (49,50) cancer tissues and differences in PAH-DNA adduct levels by race in mononuclear cells have been reported (51), no prior studies have evaluated effects of these polymorphisms on adduct levels in human prostate cancer tissues. Therefore, in this study, we extend our earlier work (13) by evaluating the potential association between smoking and polymorphisms in genes that metabolize PAHs (CYP1A1 Ile^{462}Val, CYP1B1 Ala^{119}Ser and Leu^{432}Val, mEH Tyr^{113}His and His^{139}Arg, CYP3A4 A(−392)G) and detoxify their reactive derivatives (GSTM1 null deletion, GSTP1 Ile^{105}Val) and PAH-DNA adduct levels in tumor and adjacent nontumor prostate cells in 400 men with prostate cancer.

Materials and Methods

Study Population

The study design and population have been described elsewhere (13). Briefly, the study population was composed of men from a larger case (n = 637) and control (n = 244) study who were diagnosed with prostate cancer and underwent radical prostatectomy (n = 395; 62.0%) or transure-thral resection (n = 5; 0.7%) for treatment within the Henry Ford Health System, a network of facilities comprising an 800-bed hospital in the City of Detroit, Michigan, three smaller hospitals in surrounding suburbs, and 31 medical clinics located throughout the Metropolitan Detroit area. Potential cases that indicated primary adenocarcinoma of the prostate were identified through the Henry Ford Health System pathology reports. Two of the 395 cases used in this study were initially enrolled as controls. Cases were eligible for the larger case-control study if they used the Henry Ford Health System as their primary source of health care, lived in the study area at time of recruitment (2001-2004), and had no previous history of prostate cancer.

Subjects who agreed to participate were also asked to complete a two-part interviewer-administered risk factor questionnaire (the first part was conducted over the phone; the second part was done in person) and donate a blood sample. All study protocols were approved by the Henry Ford Hospital Institutional Review Board. Clinical characteristics were obtained from medical records, and demographic, general health, and habit information (age, ethnicity, smoking) were determined from the questionnaire. Alcohol was estimated from a standardized food frequency questionnaire originally developed for two studies investigating the associations of dietary supplements and cancer risk: Vitamin and Lifestyle Cohort Study (52) and Selenium and Vitamin E Cancer Prevention Trial (53).

Genotyping

Standard venipuncture was used to collect blood samples from all study participants in tubes with EDTA as an anticoagulant. Genomic DNA was extracted from buffy coats using QIAmp DNA Blood kit (Qiagen Inc., Valencia, CA). All purified DNA samples were diluted to a constant DNA concentration in 10 mmol/L Tris, 1 mmol/L EDTA buffer (pH 8).

mEH His^{139}Arg (rs2234922) and CYP1B1 Leu^{432}Val (rs1056836) were assayed by RFLP using primer and assay conditions that have been previously described (26,54). Digestion

products were separated on a 2% agarose gel. CYP1A1 Ile^{462}Val (rs1048943), CYP1B1 Ala^{119}Ser (rs1056827) and mEPHx (mEH) Tyr^{113}His (rs1051740) polymorphisms were assayed using the GenomeLab SNP-Primer Extension assay (Beckman Coulter, Fullerton, CA) and analyzed on a CEQ 8000 Genetic Analysis System (Beckman Coulter). PCR was done using the primers (Invitrogen, Carlsbad, CA) 5′-GAACTGCCACTTCAGCTG-3′ (forward) and 5′-CTGGCTGCCCAACCAGA-3′ (reverse) for CYP1A1, 5′-GTGCTGGCCACTGTGCATGT-3′ (forward) and 5′-ACACGGCACTCATGACGTTG-3′ (reverse) for CYP1B1, and 5′-GATCGATAAGTTCCGTTTCACC-3′ (forward) and 5′-TCATTGGACTGGATGGTGCATT-3′ (reverse) for mEH. PCR was done in a 20-μL reaction with 40 ng DNA, 20 pmol of forward and reverse primers, 1.5 mmol/L $MgCl_2$, 0.25 mmol/L deoxynucleotide triphosphate, and 1.5 units AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). For CYP1B1 amplification, 2 μL DMSO was added to each reaction. PCR conditions were 10 min at 95°C, followed by 30 cycles of 95°C for 30 s, 61°C (CYP1A1) or 60°C (CYP1B1^{119} and mEH^{113}) for 35 s, and 72°C for 1 min, followed by a 6-min extension at 72°C. PCR reactions (6 μL) were cleaned with 2 units of shrimp alkaline phosphatase (Promega, Madison, WI) and 1 unit of Exonuclease I (New England Biolabs, Ipswich, MA) for 2 h at 37°C, followed by heat inactivation at 75°C for 30 min. The SNP primer extension assay was done using the GenomeLab SNP-Primer Extension kit (Beckman Coulter) according to manufacturer's instructions. SNP interrogation primers used were 5′-ATGGGCAAGCGGAAGTGTATCGGTGAGACC-3′ (forward) and 5′-AAAGACCTCCCAGCGGGCAA-3′ (reverse) for CYP1A1 Ile^{462}Val, 5′-AAAAAGGCCCTGGTGCAGCAGGGCTCGGCCTTCGCCGACCGGCCG-3′ (forward) and 5′-AAAAAAAAAAGACACCACACGGAAGGAGGCGAAGG-3′ (reverse) for CYP1B1 Ala^{119}Ser, and 5′-AAAAAAGGTGGAGATTCTCAACAGA-3′ (forward) and 5′-AAAAAAAAAATCAATCTTAGTCTTGAAGTGAGGGT-3′ (reverse) for mEH Tyr^{113}His.

The GSTP1 Ile^{105}Val (rs947894) polymorphism was detected using the Invader assay with reagents developed by Third Wave Technologies, Inc. (Madison, WI) (55). Each plate contained the following controls for the GSTP1 codon 105: (*a*) Ile/Ile homozygous, (*b*) Ile/Val heterozygous, (*c*) Val/Val homozygous, and (*d*) a no-target blank. The GSTM1 polymorphism, which results in the presence (nondeleted) or absence (null deletion) of the enzyme, was detected by a PCR product coamplified with β-globin as a positive internal control within a multiplex PCR as previously described (42).

To ensure quality control of all genotyping results, 5% of the samples were randomly selected and genotyped by a second investigator and 1% of the samples were sequenced using a 377 ABI automated sequencer.

PAH-DNA Adduct Scoring

H&E stained slides of study cases were reviewed by the study pathologist (Adnan T. Savera) to confirm the diagnosis and to identify a paraffin block with sufficient prostate tumor and nontumor prostate tissue from the radical prostatectomy for staining. For each patient sample, we used a microtome to cut five consecutive sections (5-μmol/L thick) from the tissue block. One slide was H&E stained and examined by the study pathologist who circled separate areas of prostate tumor and nontumor prostate cell populations to be used for subsequent PAH-DNA adduct scoring. The immunohistochemical assay for PAH-DNA adducts was carried out as described previously (13,49,56). This chemical assay uses the monoclonal 5D11 antibody, which in cell culture studies has been shown to produce strongly correlated staining levels (r = 0.99; P = 0.011) with the treatment dose of benzo(*a*)pyrene diol epoxide (57,58). Consistent with our previous (13) and other prior studies (49,59) using immunohistochemical assays to measure PAH-DNA adducts, we report our results in absorbance units which provides a measure of the relative intensity of staining.

For each prostate specimen, two technicians independently scored 50 epithelial cells (five fields with 10 cells per field scored) in the two areas (tumor and nontumor) circumscribed by the study pathologist. Scored cells were selected to be representative, in terms of intensity, of the cells in the field, and the mean of the two technicians' scores was used. The dual scoring technique has proved to yield a high test-retest reliability in prostate cells (13). PAH-DNA adduct data were standardized across experiments using a series of two prostate "control" slides (taken from two separate prostate specimens provided by men with prostate cancer who underwent radical prostatectomy but were not part of the study population) that were run across all batches.

Statistical Analysis

We tested the distribution of PAH-DNA adduct levels in prostatic epithelial tumor and adjacent nontumor cells for normality using the Shapiro-Wilk test statistic. Paired *t* tests were used to determine if PAH-DNA adduct levels between tumor and nontumor cells deviated significantly from zero. Correlations between explanatory variables and PAH-DNA adduct levels in tumor and nontumor cells were calculated using the parametric Pearson or nonparametric Spearman statistic if the variable deviated from normality. We calculated genotype frequencies and tested for Hardy Weinberg equilibrium within controls (prostate cancer–free men) in the larger study within major ethnic groups. We also calculated linkage disequilibrium between CYP1B1 Ala^{119}Ser and Leu^{432}Val and mEH Tyr^{113}His and His^{139}Arg alleles using epoxide hydrolase (60). We then used generalized linear regression models to estimate the association between genotypes and PAH-DNA adduct levels in prostate tumor cells and adjacent nontumor cells in the total study population and in Caucasians and African Americans, separately. Potential confounding by other factors including smoking, alcohol, and tumor characteristics [primary and total Gleason score, tumor volume, grade, prostate specific antigen (PSA) at diagnosis] was also evaluated. Models examining interactions included main effect terms (ethnicity; smoking; polymorphism under a dominant, recessive, or additive genetic model) and a multiplicative interaction term (e.g., ethnicity × genotype). All *P* values are from two-sided tests. All analyses were undertaken with SAS (version 8.2, SAS Institute Inc., Cary, NC).

Results

Characteristics of the study population are provided in Table 1. Approximately, 52.5% of the prostate cancer cases were Caucasian patients and 44.3% were African American patients. The mean age at diagnosis was 60.2 years with African American men diagnosed at a slightly younger age than Caucasian men. Approximately, 45.7% of the cases had a total Gleason score of 7 and 19.4% had a total Gleason score of >7. Although African Americans tended to present with a higher Gleason score and have greater tumor volume than Caucasians, these differences were not statistically significant. Similar to our earlier work that used 130 (13) of the 400 specimens in the present study, the distributions of PAH-DNA adduct levels in paired tumor and adjacent nontumor prostate specimens fell into two separate highly symmetrical normal distributions. Also consistent with our previous report (13), we observed a strong correlation between adduct levels in prostate tumor and nontumor prostate cells ($r = 0.51$; $P < 0.001$) and significantly higher levels of PAH-DNA adducts in nontumor cells compared with tumor cells (mean absorbance units ± SD, 0.23 ± 0.09 versus 0.16 ± 0.06; $P < 0.001$). No statistically significant differences in adduct levels between Caucasians and African Americans were observed in tumor or nontumor prostate cells. Among controls, genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium within major ethnic groups [Caucasians, $P = 0.33$ (GSTP1 Ile^{105}Val) to $P = 0.80$ (mEH His^{139}Arg); African Americans, $P = 0.12$ (GSTP1 Ile^{105}Val) to $P = 0.71$ (CYP3A4 A(−392)G)]. We also examined linkage disequilibrium between alleles in the CYP1B1 Ala^{119}Ser and Leu^{432}Val (Caucasians, D' ≤ 0.37; African Americans, D' ≤ 0.24) and the mEH Tyr^{113}His and His^{139}Arg (Caucasians, D'

≤ 0.24; African Americans, D' ≤ 0.56) polymorphisms. Significant differences in genotype (and allele) frequencies between Caucasian and African American cases in this study were observed for several polymorphisms (Table 1).

Neither ever or current smoking nor any of the PAH metabolism or conjugation polymorphisms, when examined individually, were statistically significantly associated with PAH-DNA adduct levels in the total sample (Table 2). However, stratifying by race revealed that Caucasian ever smokers (Table 3) had significantly higher adducts than nonsmokers in tumor cells (0.1748 ± 0.0052 versus 0.1507 ± 0.0070; $P = 0.006$). Moreover, Caucasians carrying two copies of the mEH ^{139}Arg allele had decreased PAH-DNA adduct levels in tumor (0.1320 ± 0.0129 versus 0.1714 ± 0.0059; $P = 0.006$) and nontumor (0.1856 ± 0.0184 versus 0.2291 ± 0.0085; $P = 0.03$) cells. Having the A-G genotype compared with the A-A genotype of the CYP3A4(−392) promoter was also positively associated with adduct levels in Caucasian tumor (0.1970 ± 0.0148 versus 0.1648 ± 0.0044; $P = 0.04$) but not Caucasian nontumor cells. In African Americans, carrying one or two CYP1B1 ^{432}Val compared with two ^{432}Leu alleles significantly increased adduct levels in tumor cells (0.1970 ± 0.0153 versus 0.1621 ± 0.0076; $P = 0.04$ or 0.1600 ± 0.0060; $P = 0.03$). Carrying one copy of the GSTP1 ^{105}Val allele significantly decreased PAH-DNA adduct levels in Caucasian nontumor cells (0.2059 ± 0.0090 versus 0.2362 ± 0.0092; $P = 0.02$) and marginally increased adduct levels in African American nontumor cells (0.2461 ± 0.0088 versus 0.2176 ± 0.0124; $P = 0.06$).

We also tested for joint effects between ethnicity, smoking, and genotypes by including an ethnicity × smoking (or genotype) interaction term in the model (Table 3). Using Caucasians as the reference group and African Americans as the risk group, we observed significant interactions between ethnicity and ever smoking [P value for interaction term (P_{int}) = 0.02] and between ethnicity and the mEH His^{139}Arg (Arg/Arg versus His/His or His/Arg; P_{int} = 0.02) polymorphisms in tumor cells. In nontumor cells, we found significant interactions between ethnicity and the mEH His^{139}Arg (Arg/Arg versus His/His or His/Arg; $P_{int} = 0.05$) and GSTP1 Ile^{105}Val (Ile/Val or Val/Val versus Ile/Ile; $P_{int} = 0.004$) polymorphisms.

We next examined joint PAH metabolism and conjugation genotype combinations on adduct levels based upon the function of the polymorphic alleles in key steps of the PAH metabolic pathway (Table 4). For example, carrying the mEH ^{113} Tyr/Tyr and mEH ^{139}His/His or His/Arg genotype combination, which may have increased PAH-epoxide to PAH-dihydrodiol hydrolysis compared with the mEH ^{113}Tyr/His or His/His and mEH ^{139}Arg/Arg genotype combination (26), increased adducts in Caucasian tumor (0.1692 ± 0.0050 versus 0.1252 ± 0.0220; $P = 0.05$) and nontumor (0.2349 ± 0.0082 versus 0.1603 ± 0.0317; $P = 0.02$) cells (data not shown). When pairing the higher metabolizing mEH ^{113}Tyr/Tyr and mEH His/His or His/Arg genotypes with the lower conjugating GSTP1 ^{105}Ile/Ile genotype compared with the mEH ^{113} Tyr/His or His/His and mEH ^{139} Arg/Arg and GSTP1 ^{105}Ile/Val or Val/Val genotype combination, we observed a significant increase in adduct levels in the nontumor cells of all study subjects (0.2472 ± 0.0130 versus 0.1537 ± 0.0331; $P = 0.01$), but when we stratified by race, this association only remained significant in Caucasian nontumor cells (0.2625 ± 0.0157 versus 0.1433 ± 0.0351; $P = 0.01$; data not shown). Similar effects were seen when combining the mEH His^{139}Arg and GSTP1 Ile^{105}Val polymorphisms (Table 4). Finally, carrying the "high-risk" genotype combination, the faster metabolism of PAH-diols to the most reactive PAH-diol-epoxide forms (CYP1B1 ^{432}Val/Val and mEH ^{139}His/His or His/Arg) with lower capacity to conjugate these PAH-diol-epoxides (GSTP1 ^{105}Ile/Ile), was associated with significantly increased adduct levels in the nontumor cells of Caucasians (0.2363 ± 0.0132 versus 0.1920 ± 0.0157; $P = 0.05$), but decreased adduct levels in non-tumor cells of African Americans (0.2121 ± 0.0175 versus 0.3060 ± 0.0408; $P = 0.05$). Cell sizes, however, continued to diminish with increasing genotype combination complexity and models with four or more polymorphisms were not estimable.

We also examined the potential joint effects of ever (Table 5) and current smoking and PAH metabolism and conjugation genes on PAH-DNA adduct levels. Increased adducts were observed in tumor cells of Caucasian ever smokers carrying the potentially faster PAH-epoxide metabolizing mEH ^{139}His/His or His/Arg genotype compared with nonsmokers with the mEH ^{139}Arg/Arg genotype (0.1798 ± 0.0056 versus 0.1358 ± 0.0182; $P = 0.02$), and Caucasian ever smokers carrying the lower PAH-diol-epoxide conjugating GSTP1 ^{105}Ile/Ile genotype had higher adducts compared with nonsmokers with the GSTP1 ^{105}Ile/Val or Val/Val genotype (0.1783 ± 0.0071 versus 0.1538 ± 0.0088; $P = 0.03$). The mEH ^{139}His/Arg or His/His genotype association became more pronounced in Caucasian current smokers' tumor (0.1937 ± 0.0147 versus 0.1342 ± 0.0132; $P = 0.003$) and nontumor cells (0.2567 ± 0.0207 versus 0.1890 ± 0.0187; $P = 0.02$; data not shown) as did the effects of the GSTP1 ^{105}Ile/Ile genotype (0.2667 ± 0.0248 versus 0.2099 ± 0.0082; $P = 0.003$). In African Americans, nonsmokers who carried the potentially lower conjugating GSTP1 ^{105}Ile/Ile genotype compared with non-smokers with GSTP1 Ile/Val or Val/Val genotype had lower adducts in nontumor cells (0.1960 ± 0.0210 versus 0.2497 ± 0.0121; $P = 0.03$). Similar effects for GSTP1 ^{105}Ile/Ile (0.2137±0.0132 versus 0.2430±0.0082; P=0.06) were observed in African Americans when examining current smoking (data not shown).

Although cell sizes became even smaller when examining the joint effects of smoking and combinations of polymorphisms, several notable associations were observed (Table 5). Ever smokers with the faster PAH-epoxide metabolizing mEH ^{113}Tyr/Tyr and mEH ^{139}His/His or His/Arg genotype combination had higher adducts in tumor (0.1697 ± 0.0046 versus 0.1295 ± 0.0161; $P = 0.02$) and nontumor (0.2377 ± 0.0065 versus 0.1725 ± 0.0232; $P = 0.008$) cells, but when we stratified by race, this effect only remained significant in Caucasian tumor (0.1740 ± 0.0070 versus 0.1349 ± 0.0182; $P = 0.05$) and nontumor cells (0.2318 ± 0.0098 versus 0.1703 ± 0.0255; $P = 0.03$; data not shown). Caucasian ever smokers with the faster metabolizing mEH ^{139}His/His or His/Arg and lower conjugating GSTP1 Ile/Ile genotype compared with nonsmokers with the mEH ^{139}Arg/Arg and GSTP1 Ile/Val or Val/Val genotype had higher adduct levels in nontumor cells (Table 5), but this effect was only statistically significant in current smokers (0.2667 ± 0.0257 versus 0.1796 ± 0.0230; $P = 0.01$; data not shown). In Caucasians, after adjusting for smoking status carrying the putative high-risk genotype, the faster metabolism of PAH-epoxides to PAH-diol-epoxides (CYP1B1 ^{432}Val/Val and mEH ^{139}His/His or His/Arg) with lower PAH-diol-epoxide conjugation (GSTP1 ^{105}Ile/Ile), was associated with marginally increased adducts in nontumor cells compared with carriers of the CYP1B1 Leu/Leu or Leu/Val, mEH ^{139}Arg/Arg and GSTP1 Ile/Val or Val/Val genotype (0.2363 ± 0.0132 versus 0.1920 ± 0.0157; $P = 0.05$; data not shown).

Discussion

When stratifying by major ethnic group, we observed significant associations between smoking, polymorphisms in PAH metabolism [CYP1B1 Leu^{432}Val, CYP3A4 A(−392)G and mEH His^{139}Arg] and conjugation (GSTP1 Ile^{105}Val) genes and PAH-DNA adducts in tumor and adjacent nontumor prostate cells. Specifically, Caucasians who reported ever smoking had significantly increased PAH-DNA adduct levels compared with nonsmokers, but this effect was not observed in African Americans. The mEH ^{139}Arg/Arg genotype, which may potentially metabolize PAH-epoxides to PAH-dihydrodiols, more slowly (26) decreased adduct levels in both prostate tumor and nontumor prostate cells of Caucasians, but this effect was not found in African Americans. In addition, having one or two copies of the GSTP1 ^{105}Val allele, which may more effectively conjugate the most reactive PAH-diol epoxides (40,41), was inversely associated with adduct levels in the tumor cells of Caucasians. Finally, carrying the putative high-risk genotype combination, the faster metabolism of PAH-dihydrodiols to their most reactive PAH-diol-epoxide forms (CYP1B1 ^{432}Leu/Leu or Leu/Val and mEH ^{139}His/His or His/Arg) with lower capacity to conjugate these PAH-diol-epoxides

(GSTP1 ^{105} Ile/Ile), was associated with increased adduct levels in nontumor cells of Caucasians, but was associated with decreased adduct levels in non-tumor cells of African Americans.

Our results are generally biologically plausible, given the expected activity of the alleles in key steps of the PAH metabolic pathway; however, functional studies are not entirely consistent and have focused on variation in one enzyme at a time, making it difficult to anticipate how variation in multiple enzymes affects PAH-DNA adduct levels. An initial *in vitro* study reported that the mEH ^{113}Tyr/^{139}Arg combination exhibits the highest protein expression (25); however, a recent study concluded the rate of hydrolysis by mEH ^{113}Tyr/^{139}His was ~2-fold greater than that measured in the other allelic combinations (26). In our observational study, we found that prostate cancer cases, particularly Caucasians, who smoke and carry two copies of the mEH ^{113}Tyr and/or one or more ^{139}His alleles have significantly higher PAH-DNA adducts in their tumor and nontumor cells, which is consistent with the most recent functional study. Although CYP1B1 ^{432}Val (with ^{119}Ala) has slightly higher activity in metabolizing benzo(*a*)pyrene-7,8-diols and other PAH metabolites [e.g., dibenzo(*a*,*l*)pyrene-diols], it has lower activity in metabolizing parent benzo(*a*)pyrene than ^{432}Leu (with ^{119}Ala), and the presence of the CYP1B1 ^{119}Ser variant seems to enhance activity in several substrates (15). We did not find any statistically significant associations with CYP1B1 Ala^{119}Ser polymorphism alone or in combination with Leu^{432}Val. Thus, additional functional studies in prostate cells, ideally with multiple polymorphic enzyme combinations, are needed to confirm our findings.

Consistent with our prior smaller study (13), PAH-DNA adducts were higher in adjacent nontumor prostate cells compared with prostate tumor cells, irregardless of ethnicity. Similar effects have not been observed in other organs. For example, Rundle et al. (49) reported that tumor cells of breast cancer cases had slightly higher PAH-DNA adducts compared with adjacent nontumor cells. As we reported previously (13), our results suggest that adducts diminish as prostate cancer foci grow and become more poorly differentiated, which may be due, in part, to loss of estrogen receptor expression in tumor cells.

Differences between ethnic groups were not totally unexpected, because African Americans, compared with Caucasians generally present with prostate cancer at an earlier age and with more advanced disease, have higher prostate cancer mortality rates (4) and higher PAH-DNA adduct levels in mononuclear cells (51). Associations between genetic polymorphisms and PAH-DNA adducts may differ by race due to differences in PAH exposure. Although we did not find a significant difference in smoking frequency between Caucasians and African Americans, PAH-DNA adduct levels, which measure the biologically effective PAH dose, were significantly increased in tumor cells of Caucasian, but not African American, ever smokers. Further stratification by genotype after adjusting for smoking status revealed that a putative high-risk genotype combination (CYP1B1 ^{432}Val/Val and mEH ^{139}His/His or His/Arg and GSTP1 Ile/Ile) significantly increased adducts in Caucasians, but not African Americans who carried this genotype combination, suggesting that other PAH-exposure sources (e.g., diet and/or occupation) may contribute to adducts in the prostate. Furthermore, why some associations varied by cell type within an ethnic group is not entirely clear. Tumor characteristics may contribute to these varying results, and in our prior (13) and current work, PAH-DNA adduct levels tended to be lower in malignant cells, but those less-differentiated measures of tumor differentiation (primary and total Gleason score), tumor volume, tumor stage, and PSA at diagnosis into the multivariable models did not materially alter results. Alternatively, differences in the cellular microenvironment, such as aberrant methylation, may lead to differential expression of these enzymes, which are within tumor and nontumor cells and between Caucasians and African Americans, potentially affecting the importance of a polymorphism. For example, silencing of GSTP1 through hypermethylation has been observed

in prostate cancer cells but not normal cells (61), and GSTP1 hypermethylation has been observed to differ significantly between Caucasians and African Americans (62). Interestingly, smoking, which contains substantial quantities of carcinogenic PAHs (63), modifies GSTP1 methylation in prostate cancer cells with current smokers having a significantly higher frequency than former or nonsmokers (64). GSTP1 hypermethylation has been shown to modify 2-amino-1-methyl-6-phenylimi-dazo[4,5-*b*]pyridine–DNA adduct levels in the prostate (65), although in a recent study involving tumor and adjacent nontumor cells from hepatic carcinoma patients, no association between GSTP1 hypermethylation and PAH-DNA adducts was found (66). Hypomethylation of the CYP1B1 promoter has been shown to increase its expression in prostate cancer but not normal cells (67), although reports on how CYP1B1 hypomethylation affects PAH-DNA adduct levels are lacking.

No prior studies have examined the effects of polymorphisms in PAH metabolism and conjugation genes and PAH-DNA adducts in prostate cancer cells; therefore, we can only compare our results to those from other tissues. Because immunoassay methods are more specific to PAH [BPDE and other similar structured]-DNA adducts whereas ^{32}P-postlabeling methods measure all hydrophobic DNA adducts (68) and results differ considerably between these two methods (69), we restrict our comparison to those studies using immunoassay methods similar to the one we used. In lung tumor tissue, the variant alleles of the CYP1A1 1*/2* (includes CYP1A1 Ile^{462}Val) and the GSTM1 null deletion polymorphisms have been positively associated with (+)*anti* –BPDE–DNA adduct levels, and the effect was more pronounced in individuals with both polymorphisms (48). In addition, carrying the GSTM1 null deletion has been shown to be positively associated with PAH-DNA adducts in tumor and adjacent nontumor cells obtained from breast cancer cases (49), but this effect was not found in another study (50). We did not find an effect with the GSTM1 null deletion polymorphism; however, we did observe significant individual and joint gene associations with the GSTP1 ^{105}Val allele which may be more efficient than GSTM1 in conjugating the most reactive PAH-diol-epoxides (33).

Strengths of our study include its large sample size for this type of molecular evaluation and nearly equal distribution of Caucasians and African Americans. However, even larger samples are needed for effectively evaluating joint effects, particularly those involving two or more polymorphisms. Furthermore, we have treated ethnicity as a dichotomous variable when, in fact, it is really a continuous trait given the large degree of racial admixture in the United States. Incorporating and adjusting for ancestry informative markers in the analyses would help minimize any error induced by dichotomizing and help clarify interpretation of results. To obtain a more complete understanding of the underlying mechanisms of PAH-induced DNA damage in prostate cells, future work should include prostate specimens from “healthy” men without prostate cancer and should evaluate the influence of other sources of PAH exposure and DNA methylation and repair mechanisms on PAH-DNA adduct formation.

In summary, this is the first report describing the individual and joint associations between smoking and polymorphisms in PAH metabolism and conjugation genes and PAH-DNA adduct levels in tumor and nontumor prostate cells. Our results suggest that the association between smoking and PAH-DNA adducts differs by race and is modified by common genetic variants lending further insight to potential gene-environment interactions in prostate carcinogenesis.

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Table 1

Prostate cancer study population characteristics and genotype frequencies for polymorphisms in PAH metabolism and conjugation genes

Characteristic or genotype	All subjects (*N* = 400)	Caucasians (*n* = 210)	African Americans (*n* = 177)	*P*[*]
Age (y)	60.2 (6.7)[†]	61.0 (6.4)[†]	59.2 (7.0)[†]	0.01
Total Gleason score				
<7	138 (34.9%)	71 (34.1%)	64 (36.6%)	0.76
7	181 (45.7%)	98 (47.1%)	76 (43.4%)	—
>7	77 (19.4%)	39 (18.8%)	35 (20.0%)	—
Tumor volume[‡]	21.1 (16.3)[†]	20.2 (15.8)[†]	21.5 (16.7)[†]	0.44
Tumor stage				
≤T2b	299 (75.5%)	154 (74.0%)	138 (78.9%)	0.27
≥T2c	97 (24.5%)	54 (26.0%)	37 (21.1%)	—
PSA at diagnosis	7.05 (6.26)[†]	7.02 (6.81)[†]	6.98 (4.97)[†]	0.94
Ever smoker	252 (63.0%)	136 (64.7%)	109 (61.6%)	0.51
Current smoker	43 (10.8%)	21 (10.0%)	20 (11.3%)	0.68
Alcohol (g)	11.91 (24.78)[†]	13.41 (24.74)[†]	10.69 (25.56)[†]	0.29
PAH-DNA adducts levels[§]				
Prostate tumor cells	0.16 (0.06)[†]	0.17 (0.06)[†]	0.16 (0.06)[†]	0.59
Prostate nontumor cells	0.23 (0.09)[†]	0.22 (0.09)[†]	0.23 (0.09)[†]	0.15
PAH metabolism genes				
CYP1A1 ^{462}Ile/Ile	358 (95.7%)	183 (94.3%)	163 (97.0%)	0.21
CYP1A1 ^{462}Ile/Val	16 (4.3%)	11 (5.7%)	5 (3.0%)	—
CYP1A1 ^{462}Val/Val	0	0	0	—
CYP1B1 ^{119}Ala/Ala	159 (42.7%)	109 (55.9%)	44 (26.8%)	<0.01
CYP1B1 ^{119}Ala/Ser	158 (42.5%)	68 (34.8%)	84 (51.2%)	—
CYP1B1 ^{119}Ser/Ser	55 (14.8%)	18 (9.2%)	36 (22.0%)	—
CYP1B1 ^{432}Leu/Leu	87 (21.9%)	66 (31.7%)	16 (9.1%)	<0.01
CYP1B1 ^{432}Leu/Val	164 (41.3%)	98 (47.1%)	61 (34.7%)	—
CYP1B1 ^{432}Val/Val	146 (36.8%)	44 (21.2%)	99 (56.2%)	—
CYP3A4 (-392)A/A	225 (57.7%)	186 (89.9%)	32 (18.8%)	<0.01
CYP3A4 (-392)A/G	94 (24.1%)	16 (7.7%)	75 (44.1%)	—
CYP3A4 (-392)G/G	71 (18.2%)	5 (2.4%)	63 (18.8%)	—
mEH ^{113}Tyr/Tyr	194 (55.3%)	88 (48.9%)	99 (62.2%)	0.02
mEH ^{113}Tyr/His	136 (38.7%)	77 (42.8%)	54 (40.0%)	—
mEH ^{113}His/His	21 (6.0%)	15 (8.3%)	6 (3.8%)	—
mEH ^{139}His/His	188 (49.6%)	106 (53.3%)	72 (43.1%)	0.11
mEH ^{139}His/Arg	150 (39.6%)	71 (35.7%)	77 (46.1%)	—
mEH ^{139}Arg/Arg	41 (10.8%)	22 (11.1%)	18 (10.8%)	—
PAH conjugation genes				
GSTM1 nondeleted[‖]	214 (61.0%)	104 (49.5%)	133 (75.6%)	<0.01
GSTM1 null	154 (39.0%)	102 (50.5%)	43 (24.4%)	—
GSTP1 ^{105}Ile/Ile	145 (36.3%)	91 (43.3%)	49 (27.7%)	0.01
GSTP1 ^{105}Ile/Val	196 (49.0%)	92 (43.8%)	98 (55.4%)	—
GSTP1 ^{105}Val/Val	59 (14.7%)	27 (12.9%)	30 (16.9%)	—

[*] *P* value comparing Caucasians to African Americans from *t* test or χ^2 test as applicable.

[†] Mean and SD (values in parentheses) of the mean.

[‡] Expressed as a percentage of the gland with tumor.

[§] Expressed as absorbance units. Adduct levels in prostate tumor and nontumor prostate cells were strongly correlated ($r = 0.51$; $P < 0.0001$).

[‖] Includes subjects with at least one copy of the nondeleted (+) allele.

Table 2

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells for smoking and genotypes in PAH metabolism and conjugation genes

Variable/genotype	Tumor cells		Nontumor cells	
	Mean ± SE*	*P*†	Mean ± SE*	*P*†
Ever smoker				
No	0.1585 ± 0.0049	—	0.2244 ± 0.0072	—
Yes	0.1680 ± 0.0038	0.13	0.2284 ± 0.0055	0.66
Current smoker				
No	0.1632 ± 0.0033	—	0.2256 ± 0.0048	—
Yes	0.1749 ± 0.0094	0.25	0.2407 ± 0.0131	0.30
CYP1B1 Ala^{119}Ser				
Ala/Ala	0.1671 ± 0.0048	—	0.2294 ± 0.0069	—
Ala/Ser	0.1638 ± 0.0049	0.62	0.2252 ± 0.0070	0.67
Ser/Ser	0.1671 ± 0.0083	0.99	0.2383 ± 0.0121	0.53
CYP1B1 Leu^{432}Val				
Leu/Leu	0.1663 ± 0.0064	—	0.2136 ± 0.0094	—
Leu/Val	0.1686 ± 0.0046	0.51	0.2300 ± 0.0068	0.16
Val/Val	0.1602 ± 0.0049	0.69	0.2340 ± 0.0072	0.09
CYP1A1 Ile^{462}Val				
Ile/Ile	0.1651 ± 0.0032	—	0.2284 ± 0.0047	—
Ile/Val	0.1746 ± 0.0149	0.54	0.2287 ± 0.0219	0.98
Val/Val	—‡	—	—‡	—
CYP3A4 A(-392)G				
A/A	0.1624 ± 0.0040	—	0.2227 ± 0.0059	—
A/G	0.1737 ± 0.0062	0.12	0.2347 ± 0.0091	0.27
G/G	0.1598 ± 0.0072	0.76	0.2305 ± 0.0105	0.52
mEH Tyr^{113}His				
Tyr/Tyr	0.1699 ± 0.0043	—	0.2383 ± 0.0063	—
Tyr/His	0.1695 ± 0.0051	0.96	0.2246 ± 0.0075	0.16
His/His	0.1786 ± 0.0129	0.52	0.2500 ± 0.0189	0.56
mEH His^{139}Arg				
His/His	0.1687 ± 0.0045	—	0.2272 ± 0.0064	—
His/Arg	0.1660 ± 0.0049	0.68	0.2227 ± 0.0071	0.57
Arg/Arg	0.1521 ± 0.0096	0.12	0.2220 ± 0.0138	0.73
GSTM1 null deletion				
+/− or +/+	0.1631 ± 0.0039	—	0.2283 ± 0.0057	—
−/−	0.1694 ± 0.0048	0.31	0.2275 ± 0.0071	0.94
GSTP1 Ile^{105}Val				
Ile/Ile	0.1613 ± 0.0050	—	0.2283 ± 0.0073	—
Ile/Val	0.1650 ± 0.0043	0.57	0.2264 ± 0.0063	0.84
Val/Val	0.1709 ± 0.0078	0.30	0.2250 ± 0.0114	0.81

NOTE: All analyses were adjusted for age, smoking, alcohol, ethnicity, Gleason score, tumor stage, and PSA at diagnosis.

*Mean absorbance units and SE of mean.

†*P* value for tests comparing wild-type/variant to wild-type/wild-type and variant/variant to wild-type/wild-type. *P* value shown is not corrected for multiple tests.

‡Not estimated because no subjects had this genotype.

Table 3

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells by major ethnic group for smoking and genotypes in PAH metabolism and conjugation genes

Variable/genotype	Caucasians				African Americans				Tumor cells, P_{int}*	Nontumor cells, P_{int}*
	Tumor cells		Nontumor cells		Tumor cells		Nontumor cells			
	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*		
Ever smoker										
No	0.1507 ± 0.0070	—	0.2138 ± 0.0102	—	0.1690 ± 0.0073	—	0.2363 ± 0.0106	—	—	—
Yes	0.1748 ± 0.0052	0.006	0.2243 ± 0.0075	0.41	0.1607 ± 0.0058	0.37	0.2349 ± 0.0085	0.92	0.02	0.67
Current smoker										
No	0.1648 ± 0.0045	—	0.2201 ± 0.0065	—	0.1603 ± 0.0062	—	0.2341 ± 0.0070	—	—	—
Yes	0.1848 ± 0.0140	0.16	0.2367 ± 0.0191	0.42	0.1628 ± 0.0130	0.86	0.2395 ± 0.0190	0.80	0.22	0.53
CYP1B1 $Ala^{119}Ser$										
Ala/Ala	0.1681 ± 0.0058	—	0.2258 ± 0.0084	—	0.1657 ± 0.0091	—	0.2357 ± 0.0131	—	—	—
Ala/Ser	0.1665 ± 0.0073	0.86	0.2198 ± 0.0106	0.66	0.1644 ± 0.0066	0.91	0.2332 ± 0.0095	0.88	—	—
Ser/Ser	0.1763 ± 0.0146	0.61	0.2298 ± 0.0213	0.86	0.1625 ± 0.0103	0.81	0.2425 ± 0.0149	0.74	0.72†	0.58†
CYP1B1 $Leu^{432}Val$										
Leu/Leu	0.1578 ± 0.0074	—	0.2121 ± 0.0108	—	0.1970 ± 0.0153	—	0.2167 ± 0.0227	—	—	—
Leu/Val	0.1736 ± 0.0060	0.10	0.2332 ± 0.0088	0.13	0.1621 ± 0.0076	0.04	0.2264 ± 0.0113	0.70	—	—
Val/Val	0.1608 ± 0.0091	0.80	0.2078 ± 0.0133	0.80	0.1600 ± 0.0060	0.03	0.2434 ± 0.0089	0.27	0.81†	0.18†
CYP1A1 $Ile^{462}Val$										
Ile/Ile	0.1674 ± 0.0045	—	0.2232 ± 0.0066	—	0.1644 ± 0.0047	—	0.2354 ± 0.0069	—	—	—
Ile/Val‡	0.1761 ± 0.0182	0.64	0.2068 ± 0.0267	0.55	0.1713 ± 0.0268	0.80	0.2769 ± 0.0389	0.29	0.94§	0.22§
CYP3A4 A(−392)G										
A/A	0.1648 ± 0.0044	—	0.2196 ± 0.0065	—	0.1589 ± 0.0108	—	0.2534 ± 0.0157	—	—	—
A/G	0.1970 ± 0.0148	0.04	0.2367 ± 0.0219	0.45	0.1683 ± 0.0069	0.46	0.2327 ± 0.0101	0.27	—	—
G/G	0.1246 ± 0.0266	0.14	0.1898 ± 0.0392	0.45	0.1622 ± 0.0076	0.81	0.2326 ± 0.0111	0.28	0.15§	0.16§
mEH $Tyr^{113}His$										
Tyr/Tyr	0.1711 ± 0.0063	—	0.2258 ± 0.0094	—	0.1698 ± 0.0061	—	0.2493 ± 0.0086	—	—	—
Tyr/His	0.1730 ± 0.0067	0.84	0.2256 ± 0.0101	0.98	0.1672 ± 0.0082	0.80	0.2266 ± 0.0117	0.12	—	—
His/His	0.1917 ± 0.0152	0.21	0.2496 ± 0.0229	0.34	0.1457 ± 0.0246	0.34	0.2510 ± 0.0351	0.96	0.33§	0.16§
mEH $His^{139}Arg$										
His/His	0.1714 ± 0.0059	—	0.2291 ± 0.0085	—	0.1675 ± 0.0071	—	0.2260 ± 0.0103	—	—	—
His/Arg	0.1724 ± 0.0072	0.92	0.2292 ± 0.0103	0.99	0.1615 ± 0.0069	0.54	0.2372 ± 0.0100	0.44	—	—
Arg/Arg	0.1320 ± 0.0129	0.006	0.1856 ± 0.0184	0.03	0.1782 ± 0.0146	0.51	0.2692 ± 0.0212	0.07	0.02†	0.05†
GSTM1 Null Deletion										
+/− or +/+	0.1622 ± 0.0059	—	0.2149 ± 0.0087	—	0.1646 ± 0.0052	—	0.2394 ± 0.0076	—	—	—
−/−	0.1737 ± 0.0059	0.16	0.2292 ± 0.0087	0.27	0.1641 ± 0.0093	0.96	0.2257 ± 0.0135	0.38	0.44	0.22
GSTP1 $Ile^{105}Val$										
Ile/Ile	0.1633 ± 0.0064	—	0.2362 ± 0.0092	—	0.1608 ± 0.0086	—	0.2176 ± 0.0124	—	—	—
Ile/Val	0.1693 ± 0.0063	0.50	0.2059 ± 0.0090	0.02	0.1615 ± 0.0061	0.95	0.2461 ± 0.0088	0.06	—	—
Val/Val	0.1665 ± 0.0116	0.81	0.2193 ± 0.0166	0.37	0.1775 ± 0.0111	0.24	0.2296 ± 0.0161	0.56	0.96§	0.004§

NOTE: All analyses were adjusted for age, smoking, alcohol, Gleason score, tumor stage, and PSA at diagnosis.

* *P* value for multiplicative interaction term: smoking or genotype × ethnic group.

† *P* value for interaction term (gene × race) under a recessive genetic model.

‡ Val/Val not estimated because no subjects had this genotype.

§ P value for interaction term (gene × race) under a dominant genetic model.

Table 4

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells among all study subject and within major ethnic group for genotype combinations of PAH metabolism and conjugation genes

Genotype combination	All subjects					Caucasians					African Americans				
	n	Tumor		Nontumor		*n*	Tumor		Nontumor		*n*	Tumor		Nontumor	
		Mean ± SE	*P*	Mean ± SE	*P*		Mean ± SE	*P*	Mean ± SE	*P*		Mean ± SE	*P*	Mean ± SE	*P*
CYP1B1 Leu^{432}Val and GSTP1 Ile^{105}Val:															
Lower metabolism and higher conjugation:* CYP1B1 Leu/Leu or Leu/Val and GSTP1 Ile/Val or Val/Val	159	0.1679 ± 0.0047	—	0.2179 ± 0.0065	—	96	0.1688 ± 0.0063	—	0.1980 ± 0.0408	—	57	0.1695 ± 0.0080	—	0.2275 ± 0.0112	—
Faster metabolism and lower conjugation:* CYP1B1 Val/Val and GSTP1 Ile/Ile	52	0.1570 ± 0.0083	0.26	0.2218 ± 0.0115	0.77	22	0.1589 ± 0.0134	0.51	0.2474 ± 0.0078	0.66	29	0.1560 ± 0.0111	0.33	0.2187 ± 0.0155	0.64
mEH His^{139}Arg and GSTP1 Ile^{105}Val:															
Lower metabolism and higher conjugation:* mEH Arg/Arg and GSTP1 Ile/Val or Val/Val	31	0.1537 ± 0.0108	—	0.2316 ± 0.0080	—	16	0.1332 ± 0.0135	—	0.1754 ± 0.0222	—	15	0.1626 ± 0.0105	—	0.2107 ± 0.1338	—
Faster metabolism and lower conjugation:* mEH His/His or His/Arg and GSTP1 Ile/Ile	126	0.1630 ± 0.0053	0.08	0.2150 ± 0.0162	0.36	81	0.1651 ± 0.0061	0.04	0.2451 ± 0.0100	0.005	42	0.1771 ± 0.0182	0.49	0.2603 ± 0.0238	0.08
CYP1B1 Leu^{432}Val and mEH His^{139}Arg and GSTP1 Ile^{105}Val:															
Lower metabolism and higher conjugation:* mEH Arg/Arg and CYP1B1 Leu/Leu or Leu/Val & GSTP1 Ile/Val or Val/Val	18	0.1497 ± 0.0166	—	0.2256 ± 0.0198	—	18	0.1354 ± 0.0133	—	0.1920 ± 0.0157	—	6	0.1840 ± 0.0321	—	0.3060 ± 0.0408	—
Faster metabolism and lower conjugation:* mEH His/His or His/Arg and CYP1B1 Val/Val & GSTP1 Ile/Ile	46	0.1587 ± 0.0102	0.65	0.2236 ± 0.0122	0.93	18	0.1658 ± 0.0111	0.10	0.2363 ± 0.0132	0.05	27	0.1547 ± 0.0138	0.41	0.2121 ± 0.0175	0.05

NOTE: All analyses were adjusted for age, smoking, alcohol, Gleason score, tumor stage, and PSA at diagnosis. Analyses in the total sample were also adjusted for ethnicity.

* Putative metabolism and conjugation combinations (see text for details).

Table 5

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells in major ethnic groups for select genotypes and genotype combinations of PAH metabolism and conjugation genes by ever smoker status

Genotype	Caucasians										African Americans									
	Tumor						Nontumor				Tumor						Nontumor			
	Nonsmoker			Smoker			Nonsmoker		Smoker		Smoker			Nonsmoker			Smoker		Nonsmoker	
	*n**	Mean ± SE	*P*	*n**	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*	*n**	Mean ± SE	*P*	*n**	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*
CYP1B1 Leu/Leu or Leu/Val	145	0.1506 ± 0.0079	—	42	0.1763 ± 0.0058	0.01	0.2192 ± 0.0116	—	0.2278 ± 0.0085	0.55	70	0.1718 ± 0.0075	—	86	0.1597 ± 0.0059	0.43	0.2432 ± 0.0114	—	0.2393 ± 0.0090	0.77
CYP1B1 Val/Val	19	0.1522 ± 0.0154	0.93	2	0.1654 ± 0.0113	0.28	0.1977 ± 0.0225	0.40	0.2132 ± 0.0165	0.77	7	0.2285 ± 0.0286	0.42	13	0.2026 ± 0.0202	0.17	0.2306 ± 0.0433	0.24	0.2242 ± 0.0306	0.31
mEH ^{139}Arg/Arg	11	0.1358 ± 0.0182	—	11	0.1281 ± 0.0181	0.77	0.1971 ± 0.0261	—	0.1739 ± 0.0261	0.53	7	0.1859 ± 0.0228	—	11	0.1729 ± 0.0191	0.66	0.2790 ± 0.0331	—	0.2624 ± 0.0277	0.71
mEH ^{139}His/His or His/Arg	59	0.1557 ± 0.0079	0.31	118	0.1798 ± 0.0056	0.02	0.2235 ± 0.0114	0.36	0.2320 ± 0.0080	0.20	58	0.1704 ± 0.0079	0.52	91	0.1606 ± 0.0064	0.29	0.2331 ± 0.0115	0.19	0.2309 ± 0.0092	0.16
GSTP1 Ile/Val or Val/Val	47	0.1538 ± 0.0088	—	72	0.1783 ± 0.0071	0.03	0.2128 ± 0.0126	—	0.2064 ± 0.0102	0.69	51	0.1756 ± 0.0083	—	77	0.1581 ± 0.0068	0.10	0.2497 ± 0.0121	—	0.2373 ± 0.0100	0.43
GSTP1 Ile/Ile	27	0.1450 ± 0.0118	0.55	64	0.1708 ± 0.0076	0.14	0.2157 ± 0.0169	0.89	0.2447 ± 0.0109	0.06	17	0.1492 ± 0.0145	0.12	32	0.1669 ± 0.0105	0.52	0.1960 ± 0.0210	0.03	0.2292 ± 0.0153	0.30
CYP1B1 Leu/Leu or Leu/Val and GSTP1 Ile/Val or Val/Val	87	0.1540 ± 0.0099	—	20	0.1790 ± 0.0082	0.05	0.2220 ± 0.0133	—	0.2063 ± 0.0110	0.36	52	0.1811 ± 0.0136	—	25	0.1635 ± 0.0098	0.30	0.2381 ± 0.0193	—	0.2220 ± 0.0139	0.50
CYP1B1 Val/Val and GSTP1 Ile/Ile	9	0.1518 ± 0.0233	0.93	2	0.1625 ± 0.0165	0.66	0.2316 ± 0.0313	0.78	0.2165 ± 0.0221	0.83	5	0.1388 ± 0.0188	0.07	4	0.1651 ± 0.0136	0.41	0.2127 ± 0.0267	0.44	0.2218 ± 0.0193	0.55
mEH ^{139} Arg/Arg and GSTP1 Ile/Val or Val/Val	8	0.1391 ± 0.0192	—	8	0.1274 ± 0.0193	0.67	0.2033 ± 0.0317	—	0.1476 ± 0.0318	0.22	6	0.1885 ± 0.0280	—	9	0.1686 ± 0.0243	0.59	0.2754 ± 0.0355	—	0.2490 ± 0.0307	0.58
mEH ^{139}His/His or His/Arg and GSTP1 Ile/Ile	22	0.1490 ± 0.0119	0.66	59	0.1709 ± 0.0071	0.12	0.2364 ± 0.0196	0.38	0.2482 ± 0.0118	0.19	15	0.1688 ± 0.0132	0.53	27	0.1514 ± 0.0177	0.27	0.1898 ± 0.0224	0.05	0.2224 ± 0.0167	0.18

NOTE: All analyses were adjusted for age, smoking, alcohol, Gleason score, tumor stage, and PSA at diagnosis. Reference group includes nonsmokers with the putative lower metabolizing, higher conjugating or lower metabolizing and higher conjugating genotype combination.

*Sample size is the same for tumor and nontumor cells within the same major ethnic genotype by smoking group; thus, sample size is only listed once under the tumor cell heading.

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Racial differences in clinical and pathological associations with PhIP-DNA adducts in prostate

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Abstract

African–American men have a higher dietary intake of 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), which is the most abundant heterocyclic amine in cooked meats and is carcinogenic in rat prostate through the formation of DNA adducts. To determine the clinical and demographic factors associated with PhIP-DNA adduct levels, the biologically effective dose of PhIP in human prostate, we immunohistochemically measured PhIP-DNA adducts in a study of 162 Caucasian and 102 African–American men who underwent radical prostatectomy for prostate cancer. A strong correlation between PhIP-DNA adduct levels in prostate tumor and adjacent non-tumor cells was observed ($\rho = 0.62$; $p < 0.0001$); however, non-tumor cells had significantly higher adduct levels compared with tumor (0.167 optical density (OD) units ± 0.043 *vs.* 0.104 OD ± 0.027; $p < 0.0001$). Race was not associated with PhIP-DNA adduct levels in either tumor or non-tumor cells, but race-specific associations were observed. In prostate tumor and non-tumor cells, tumor volume had the strongest association with PhIP-DNA adducts in Caucasians, whereas in African–Americans prostate volume was most strongly associated with adduct levels in tumor cells and advanced Gleason grade had the strongest association in non-tumor cells. In race interaction models, while the only statistically significant interaction was between African–American race and advanced Gleason grade in non-tumor cells ($\beta = 0.029$; $p = 0.02$), in tumor cells we observed opposite effects by race (positive for African–Americans, negative for Caucasians) for older age and high PSA levels at diagnosis. In conclusion, while PhIP-DNA adduct levels in prostate cells do not vary significantly by race, our results suggest that PhIP exposure may have stronger effects on prostate tumor differentiation in African–American men.

Keywords

2-amino-1-methyl-6-phenylimidazo(45-b)pyridine; African–Americans; immunohistochemistry; neoplasms; DNA damage

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Dietary sources of potential prostate carcinogens include fat and meat.[1] One possible link between a diet high in meat consumption and prostate cancer is the cooking of meat at high temperatures and the subsequent formation of heterocyclic amines (HA), which are potent carcinogens in animals.[2,3] A direct correlation between HA exposure and DNA adduct formation in the prostate is supported by animal studies and *in vitro* studies of human tissues. Rats given food-derived 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the most abundant HA in human diets, for 52 weeks had PhIP-DNA adducts in all prostate lobes and subsequently developed prostate cancer.[3] The tumorigenic potential of PhIP-laden diets in rats has also been shown by significantly elevated mutation frequencies in all prostate lobes after only 4 weeks[4] and a significant prostate tumor incidence within 20 weeks.[5] Nude mice administered an intragastric injection of PhIP showed positive staining for PhIP-DNA adducts in 70–95% of both epithelial and stromal cells in human prostate xenografts.[6] Several *in vitro* studies of human prostatic tissue incubated in HA laden milieu have demonstrated detectable PhIP-DNA adducts in prostate cells afterwards,[7-9] but one study that examined human prostate specimens that were not experimentally exposed to PhIP found only 2 of 24 specimens had detectable PhIP-DNA adducts.[10] *In vitro* experiments of human prostate epithelial cells have shown that increased doses of PhIP result in increased DNA damage as measured by the comet assay.[11]

Metabolic activation of PhIP, which is necessary for DNA adduct formation, is thought to occur primarily in the liver *via* a two-step process in which N-oxidation of the PhIP compound is catalyzed by cytochrome P4501A2 (CYP1A2). Subsequent acetylation or sulfation is catalyzed by acetyltransferases (NAT) or sulfotransferases (SULT), respectively, which generate *N*-acetoxy or *N*-sulfonyloxy esters, electrophiles that are much more reactive with DNA.[12,13] Recent studies have shown that this two-step bioactivation of PhIP may also occur in the prostate. PhIP-DNA adducts formed at levels 30–600 times higher when human prostate tissues were incubated with *N*-hydroxy PhIP compared with PhIP.[8] Several studies have demonstrated expression of CYP1A2 in prostate cells[8,14,15] (previously thought to be confined to the liver), which supports the *O*-acetylation step of PhIP also occurring in the prostate. In addition, Nelson *et al*. showed that loss of expression of an important gatekeeper in the prostate carcinogenic pathway, GSTP1, enhances susceptibility to carcinogenic insult by *N*-OH-PhIP in prostate.[9]

The U.S. population lifetime time-weighted average of total HA consumed has been estimated to be ~9 ng/kg/day, with PhIP comprising about two thirds of this intake.[16] Mean HA intakes are greatest for African–American males, who were estimated to consume ~2- to 3-fold more PhIP than their white male counterparts.[16] Biomarker studies support the findings from dietary questionnaires in that African–American men have been shown to excrete a higher level of HA metabolites.[17,18] In addition to having a greater HA exposure, African–American men may be at greater biologic risk due to higher activity levels of the human sulfotransferase 1A1 (SULT1A1) enzyme that is involved in the bioactivation of *N*-hydroxy metabolite of PhIP.[13] Although race-specific prostate cancer risk associated with SULT1A genotypes are comparable, African–American men with the highest human SULT1A1 activity levels are at a 2-fold greater risk for prostate cancer compared with Whites at comparable SULT1A1 activity levels,[19] which suggests that additional factors interacting with SULT1A1 may increase prostate cancer risk in African–Americans.

Despite evidence that PhIP-DNA adducts may play an important role in prostate carcinogenesis, population level studies of PhIP-DNA adducts in the prostate do not exist. Such studies are necessary to determine whether HA intake results in a biologically effective dose measurable in the prostate and whether PhIP-DNA adducts might explain racial differences in prostate cancer risk and outcomes. In the present study, we describe the distribution of PhIP-DNA adduct levels in tumor and non-tumor prostate cells of prostate cancer cases who underwent radical prostatectomy, investigate potential associations between clinical and

histologic characteristics and PhIP-DNA adduct levels, and test whether race is a modifying factor for these associations.

Material and methods

Study Sample

The study population consisted of men who were part of the Henry Ford Health System. Details concerning the ascertainment and recruitment of study cases can be found in a previous publication.[20] The present study includes 264 men who underwent radical prostatectomy for prostate cancer. Slides were cut from a specimen block that contained both tumor and non-tumor cells and subject to immunohistochemical studies for PhIP-DNA adduct determination. The analytic sample was 39% African–American and had a mean age at diagnosis of 60.9 ± 6.9 years with an average of 3 ± 2.5 months between diagnosis and surgery.

Pathology

Hematoxylin-eosin stained slides of study cases were reviewed by the study uropathologist (ATS) to confirm the diagnosis and identify a paraffin block with sufficient tumor and non-tumor prostatic tissue for staining. Using a microtome, 5 consecutive sections (5 μm thick) were cut from the tissue block of each patient sample. One slide was hematoxylin and eosin stained and examined by the study uropathologist who circled separate areas of tumor and non-tumor cell populations to be used for adduct scoring. Tumors were given a TNM staging score and characterized by histologic grade (*i.e.*, primary and secondary Gleason scores), lobe involvement, volume, resection margins, vascular invasion, perineural invasion, extraprostatic extension, and seminal vesicle and lymph node involvement. Prostate volumes were also calculated based on the dimensions of the gland measured after surgical removal.

Immunohistochemistry

Immunohistochemistry studies were performed as described by Takahashi *et al.*[21] and Zhu *et al.*[22,23] Briefly, the paraffin-embedded sections were baked at 59°C for 1 hr, deparaffinized in xylene, and rehydrated in serial alcohol. Endogenous peroxidase activity was blocked using 0.3% H_2O_2 in methanol for 20 min. After treatment using RNase and proteinase K, the sections were blocked using 3% BSA and normal goat serum. The primary anti-PhIP-DNA adduct polyclonal antibody was provided by Dr. Shirai at Nagoya City University Medical School, Nagoya, Japan.[3] The polyclonal antibody was incubated with the sections at 4°C overnight in a humid chamber at a dilution of 1:750. The biotinylated secondary antibody was incubated with the sections at room temperature for 30 min, at a dilution of 1:200. The antibody complex was detected using an avidin-biotin-peroxidase complex solution and visualized using 3,3′-diaminobenzidine (Zymed Laboratories, San Francisco, CA). The staining specificity was confirmed with positive control samples that were run with every experimental batch using the primary antibody preabsorbed with 2 or 20 μg/mL DNA extract from MCF-7 cells and treated with 150 μM *N*-hydroxy-PhIP. A cytospin sample of MCF-7 cells without PhIP treatment served as a negative exposure control. In addition, slides of prostate tumor from a non-study subject were inserted into each batch of staining in which the primary anti-PhIP-DNA adduct polyclonal antibody was omitted (negative control) and the specimen was treated with both antibodies (positive control). The scores of the positive controls also served as calibration references between staining batches. Staining was quantified by absorbance image analysis in optical density (OD) units using a Cell Analysis System 200 microscope as described previously.[24]

Statistical analyses

PhIP-DNA adduct levels in prostatic epithelial tumor and non-tumor were tested for normality using the Shapiro-Wilk statistic. Paired *t*-tests were used to discern whether the difference in adduct levels between tumor and non-tumor cells deviated significantly from zero. Multiple linear regression models were used to calculate group specific means and standard errors and test for associations between predictor and outcome variables. Potential batch effects in the PhIP-DNA adduct assay were taken into account by computing a batch correction factor that was the difference between the adduct level of the positive control slide in a single batch and the mean adduct level of the positive control slides across all batches. The batch-adjusted adduct level was the crude adduct level minus the batch correction factor. This approach was used in our previous studies involving PAH-DNA adducts.[24] Statistical significance was assessed at the Type-I error level of 0.05 and all tests were two-sided.

Results

The distributions of PhIP-DNA adduct levels in 264 paired tumor and non-tumor prostate specimens are shown in Figure 1. Both the tumor and non-tumor PhIP-DNA adduct distributions were highly symmetrical based on a Shapiro-Wilk goodness-of-fit statistic. PhIP-DNA adduct levels in tumor and non-tumor prostate cells fell into 2 separate normal distributions with the mean level of adducts in non-tumor cells 0.063 OD units significantly higher than that in tumor cells (O.167 ± 0.043 OD *vs*. 0.104 ± 0.027 OD; $p < 0.0001$). A strong correlation between adduct levels in tumor and non-tumor cells was observed ($\rho = 0.62$; $p < 0.0001$) with the absolute difference in adduct levels between the 2 cell types constant across all levels based on a slope of one from a linear regression model (Fig. 2).

Table I depicts the mean PhIP-DNA adduct levels in prostate tumor cells for all subjects and within each racial group. In all subjects, PhIP-DNA adduct levels were higher in tumors that involved less than 20% of the prostate and in smaller prostates. Examining these 2 factors together, the mean adduct level in smaller prostates with low tumor volume was 23% higher than the mean adduct level in larger prostates with high tumor volume (0.116 *vs.* 0.094 OD). While the direction of these associations was consistent between Caucasians and African–Americans, a statistically stronger association with adduct levels and tumor volume was observed in Caucasians whereas prostate volume had a greater statistical association with adduct level in African–Americans. A weak ($p = 0.1$) positive association between advanced Gleason grade and adduct level was also observed exclusively in African–Americans. In non-tumor cells (Table II), tumor volume was inversely associated with PhIP-DNA adduct level in Caucasians with a weaker, but similar, association observed in African–Americans. In tumor cells, prostate volume was not significantly associated with adduct level in either race, and only in African–Americans was an association between advanced Gleason grade and adduct level observed ($p = 0.005$).

We next used multiple linear regression analyses to estimate race-specific beta coefficients for the independent associations of the clinical parameters of interest with PhIP-DNA adduct level in prostate tumor and non-tumor cells (Table III). Tumor volume was inversely associated with PhIP-DNA adduct level in both tumor and non-tumor cells in both races, but this association reached greater statistical significance in the Caucasian sample. In African–Americans, an inverse association between tumor volume and adduct level in tumor and non-tumor cells was also observed, but it was not as strong as in Caucasians. In tumor cells, prostate volume had a stronger inverse association with adduct levels in African–Americans. Age, PSA level and advanced Gleason grade were all positively associated with adduct level in tumor and non-tumor cells in African–Americans, with advanced Gleason grade in non-tumor cells having the largest and most statistically significant β coefficient ($\beta = 0.029 \pm 0.012$; $p = 0.02$). The African–American race by advanced Gleason grade interaction term in non-tumor cells was

also the largest and most statistically significant (β = 0.029 ± 0.013; p=0.02). In tumor cells, other notable interactions with African–American race were observed for older (60+) age (β = 0.012 ± 0.017; p = 0.08) and the highest PSA level category (>10 ng/mL) at diagnosis (β = 0.019 ± 0.012; p = 0.12).

In a related study, we found PhIP-DNA adduct levels were strongly correlated with grilled meat consumption and in particular grilled red meat consumption.[25] Racial differences in grilled meat consumption in our study population were mixed. For specific meats, Caucasian men had significantly higher consumption of steak (p = 0.035), hamburger (p < 0.001), and chicken without skin (p < 0.001), while African–American men had significantly higher consumption of chicken with skin (p = 0.006). Overall grilled meat consumption was slightly higher in Caucasians (p = 0.045) as was grilled red meat consumption (p = 0.037).

Figure 3*a-c* shows representative tumor and non-tumor prostate cells from tumors of primary Gleason Grade 3 and 4. By visual inspection, one can see the dark staining for PhIP-DNA adducts in non-tumor prostate cells (Panel A), the relative absence of staining for PhIP-DNA adducts in tumor cells of primary Gleason 3 (Panel B) and mixed light and dark staining for PhIP-DNA adducts in tumor cells of primary Gleason Grade 4 (Panel C).

Discussion

In an unselected sample of men who underwent radical prostatectomy for treatment of their prostate cancer, variations in PhIP-DNA adduct levels were measurable in their tumor and non-tumor prostate cells. Previous *in vitro* studies have shown that human prostate tissue can metabolically activate “cooked meat” carcinogens, including PhIP.[8,26] PhIP-DNA adduct levels were significantly lower in tumor cells compared with that in non-tumor cells consistent with what we previously observed in this same population for PAH-DNA adducts.[24] This difference might be caused by higher cell turnover in tumor cells. It might also be due to differences in metabolism[15] or DNA repair[27] between normal and malignant cells. Tumor cells are mostly cloned from one mutated cell, and therefore more homogeneous than non-tumor cells. Microarray studies have demonstrated that hundreds of genes are differentially expressed between prostate tumor and non-tumor cells.[28]

PhIP-DNA adduct levels were the same in African–Americans and Caucasians, even after taking into account potential confounders such as tumor stage or grade. In a parallel study, we found grilled red meat consumption was strongly associated with PhIP-DNA adduct level in prostate tumor cells and moderately associated with PhIP-DNA adduct level in prostate non-tumor cells.[25] While analysis of the grilled meat consumption variables found slightly higher consumption of both grilled red meat and all grilled meat in Caucasians, we found no racial differences in overall PhIP-DNA adduct levels in either tumor or adjacent non-tumor prostate cells. However, we did find that PhIP-DNA adduct levels had differential associations with some clinical and pathologic factors by race. An African–American race by advanced Gleason grade interaction term was significant in non-tumor cells (p = 0.02) with a similar trend of positive association between adduct level and higher Gleason grade exclusive to African–Americans observed in tumor cells. Older age and high pre-diagnosis PSA level were also two factors with moderate positive associations with adduct levels in tumor cells of African–Americans, but had no associations with adduct levels in Caucasians.

A growing body of evidence supports the notion that prostate carcinogenesis is biologically different in African–American men. Black patients who underwent radical prostatectomy in one study exhibited a higher incidence of transition zone cancer foci and higher serum PSA levels in patients with locally advanced prostate cancer.[29] In a more recent study, African–American men with organ confined disease and moderate PSA levels had higher overall tumor

volumes than white men with comparable clinical characteristics.[30] In general, African–American race has been associated with higher PSA levels at prostate cancer diagnosis.[29, 31-33] Having both a high PSA level and a poorly differentiated prostate tumor is more likely in African–Americans than Caucasians.[33] While underlying biology may be in part responsible for these observed racial differences in PSA level at prostate cancer diagnosis, a recent study of healthy African–American men found that high dietary PhIP intake was correlated with elevated PSA levels.[34] The associations between high PSA level and Gleason grade and PhIP exposure observed in African–Americans is consistent with the suggestive positive associations we found in our study between PhIP-DNA adduct levels in both tumor and non-tumor cells and high PSA levels and advanced Gleason that was exclusive to African–American men. In terms of metabolizing PhIP, African–American men with the highest human SULT1A1 activity levels, an enzyme which is involved in the bioactivation of *N*-hydroxy metabolite of PhIP,[13] are at a 2-fold greater risk for prostate cancer compared with Whites at comparable SULT1A1 levels.[19] African–Americans are also known to have higher enzymatic activity levels of CYP1A2 and *N*-acetyltransferase,[35] two enzymes important in the *O*-acetylation and N-oxidation of PhIP, respectively. Interestingly, CYP1A2 appears to be strongly inducible by smoking in whites, but not in African–Americans,[36] which has potentially important implications in the race-specific effects of PhIP exposure on cancer risk.

Our study was not designed to directly test whether PhIP-DNA adducts increase risk for prostate cancer, but rather to describe the distribution of and determine what factors influence PhIP-DNA adduct levels in prostate cells of men with prostate cancer. While previous studies have suggested that African–Americans are both exposed to higher levels of PhIP and excrete higher levels of PhIP metabolites, we found no evidence for a racial difference in PhIP-DNA adducts in either non-tumor or tumor prostate cells. Our results do suggest, however, that prostate tumor differentiation may be more strongly linked with PhIP exposure in African–Americans, which implies that the PhIP-induced carcinogenesis pathway in the prostate may biologically differ by race. Future studies of the PhIP metabolism pathway in prostate cancer should consider how race-specific factors may influence the importance of PhIP with regard to the ethnic variation in this disease.

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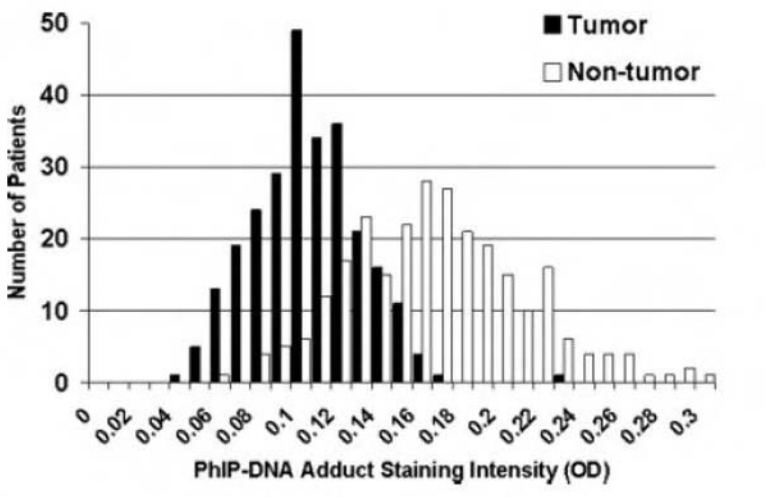

FIGURE 1.

PhIP-DNA adduct staining intensity frequency distribution in 264 prostate cancer patients based on optical density scores in tumor and adjacent non-tumor prostate cells.

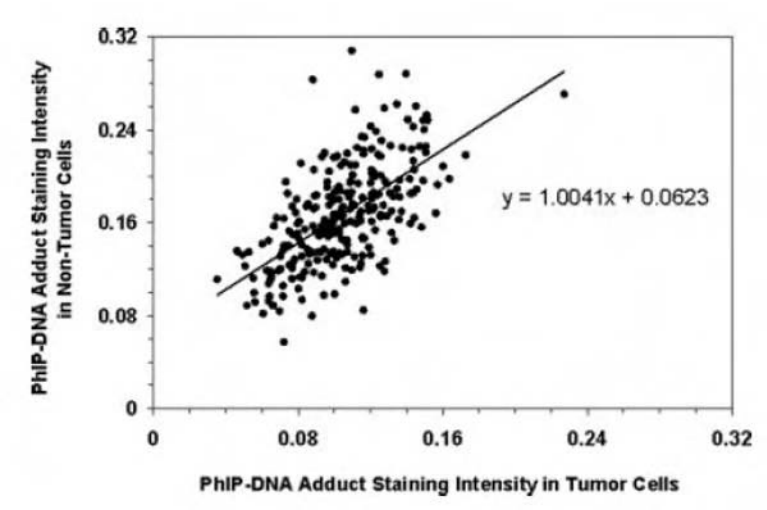

FIGURE 2.

Correlation between PhIP-DNA adduct optical density scores in tumor and adjacent non-tumor prostate cells in 264 prostate cancer patients.

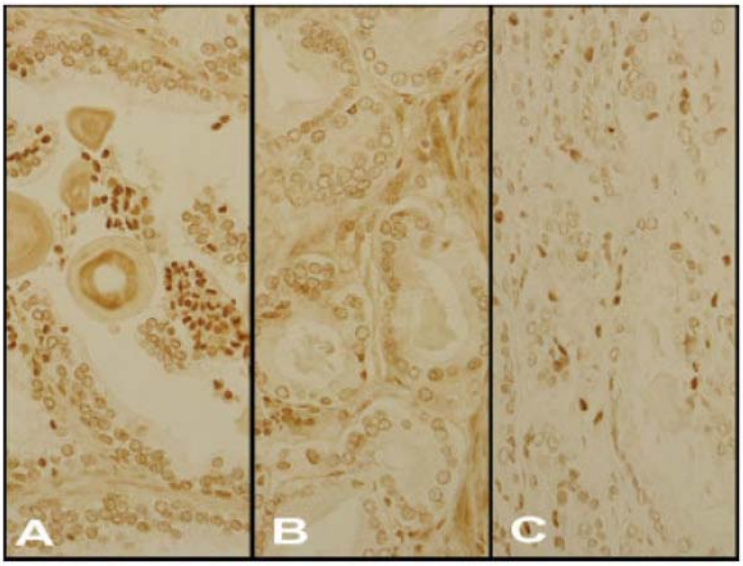

FIGURE 3.

PhIP-DNA adduct staining in prostate epithelial non-tumor (Panel A) and tumor cells of Gleason primary Grade 3 (Panel B) and 4 (Panel C). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I

MEAN PhIP-DNA ADDUCT LEVELS IN PROSTATE TUMOR CELLS EXPRESSED AS ABSORBANCE UNITS FOR ALL SUBJECTS AND STRATIFIED BY RACE

	All subjects (*n* = 264)			Caucasians (*n* = 162)			African–Americans (*n* = 102)		
	n	**Mean ± SE**	***p* value**	***n***	**Mean ± SE**	***p* value**	***n***	**Mean ± SE**	***p* value**
Age at prostatectomy									
<60	114	0.105 ± 0.003	0.91	66	0.105 ± 0.003	0.36	48	0.103 ± 0.004	0.34
60+	150	0.104 ± 0.002		96	0.102 ± 0.003		54	0.109 ± 0.004	
PSA at diagnosis									
Low (<4 ng/mL)	45	0.105 ± 0.004	0.88	34	0.106 ± 0.004	0.38	11	0.104 ± 0.008	0.53
Moderate (4–10 ng/mL)	176	0.105 ± 0.002		98	0.104 ± 0.003		78	0.105 ± 0.003	
High (>10 ng/mL)	43	0.103 ± 0.004		30	0.097 ± 0.005		13	0.114 ± 0.008	
Tumor volume									
Low (<20% of gland)	136	0.110 ± 0.002	0.0006	82	0.109 ± 0.003	0.003	54	0.111 ± 0.004	0.07
High (≥20% of gland)	128	0.099 ± 0.002		80	0.097 ± 0.003		48	0.101 ± 0.004	
Prostate volume									
Low (< 65 cc)	133	0.108 ± 0.002	0.02	78	0.105 ± 0.003	0.35	55	0.112 ± 0.004	0.02
High (≥65 cc)	131	0.101 ± 0.002		84	0.101 ± 0.003		47	0.099 ± 0.004	
Tumor stage									
2	204	0.105 ± 0.002	0.54	125	0.103 ± 0.003	0.76	79	0.107 ± 0.003	0.56
3 or 4	60	0.103 ± 0.003		37	0.102 ± 0.004		23	0.103 ± 0.004	
Advanced Gleason Grade[1]									
No	200	0.103 ± 0.002	0.31	122	0.103 ± 0.002	0.97	78	0.104 ± 0.004	0.10
Yes	64	0.107 ± 0.003		40	0.103 ± 0.004		24	0.114 ± 0.006	

[1] No = Gleason sum 6 or less or Gleason sum 7 and primary Gleason grade 3 or lower; Yes = Gleason sum 8 or higher or Gleason sum 7 and primary Gleason grade 4 or higher.

TABLE II

MEAN PhIP-DNA ADDUCT LEVELS IN PROSTATE NON-TUMOR CELLS EXPRESSED AS ABSORBANCE UNITS FOR ALL SUBJECTS AND STRATIFIED BY RACE

	All subjects (*n* = 264)			Caucasians (*n* = 162)			African–Americans (*n* = 102)		
	n	**Mean ± SE**	***p* value**	***n***	**Mean ± SE**	***p* value**	***n***	**Mean ± SE**	***p* value**
Age at prostatectomy									
<60	114	0.167 ± 0.004	0.99	66	0.169 ± 0.005	0.53	48	0.164 ± 0.007	0.47
60+	150	0.167 ± 0.004		96	0.165 ± 0.004		54	0.171 ± 0.006	
PSA at diagnosis									
Low (<4 ng/mL)	45	0.105 ± 0.004	0.88	34	0.161 ± 0.007	0.56	11	0.153 ± 0.014	0.37
Moderate (4–10 ng/mL)	176	0.105 ± 0.002		98	0.170 ± 0.004		78	0.168 ± 0.005	
High (>10 ng/mL)	43	0.103 ± 0.004		30	0.162 ± 0.008		13	0.179 ± 0.013	
Tumor Volume									
Low (<20% of gland)	136	0.173 ± 0.004	0.02	82	0.175 ± 0.005	0.02	54	0.171 ± 0.006	0.52
High (≥20% of gland)	128	0.161 ± 0.004		80	0.159 ± 0.005		48	0.165 ± 0.007	
Prostate volume									
Low (<65 cc)	133	0.171 ± 0.004	0.11	78	0.172 ± 0.005	0.11	55	0.170 ± 0.006	0.59
High (≥65 cc)	131	0.163 ± 0.004		84	0.161 ± 0.005		47	0.165 ± 0.007	
Tumor stage									
2	204	0.166 ± 0.003	0.65	125	0.167 ± 0.004	0.81	79	0.166 ± 0.005	0.31
3 or 4	60	0.169 ± 0.006		37	0.165 ± 0.007		23	0.177 ± 0.010	
Advanced Gleason Grade[1]									
No	200	0.165 ± 0.003	0.09	122	0.167 ± 0.004	0.94	78	0.161 ± 0.005	0.005
Yes	64	0.175 ± 0.005		40	0.166 ± 0.007		24	0.190 ± 0.009	

[1] No = Gleason sum 6 or less or Gleason sum 7 and primary Gleason grade 3 or lower; Yes 5 Gleason sum 8 or higher or Gleason sum 7 and primary Gleason grade 4 or higher.

TABLE III

RACE-SPECIFIC EFFECTS OF CLINICAL FACTORS ON MEAN PhIP-DNA ADDUCT LEVELS IN PROSTATE TUMOR AND NON-TUMOR CELLS[1]

	Caucasians (n = 162)		African–Americans (n = 102)		AA race × risk factor interaction	
	B ± SE	***p* value**	**β ± SE**	***p* value**	**β ± SE**	***p* value**
Older Age at prostatectomy (≥60)						
Tumor	−0.003 ± 0.004	0.43	0.009 ± 0.005	0.10	0.012 ± 0.007	0.08
Non-tumor	−0.002 ± 0.007	0.77	0.006 ± 0.009	0.52	0.013 ± 0.011	0.24
PSA at diagnosis Tumor						
Tumor						
Moderate (4–10 ng/mL)	−0.0006 ± 0.005	0.90	0.007 ± 0.009	0.42	0.006 ± 0.010	0.55
High (>10 ng/mL)	−0.005 ± 0.007	0.49	0.018 ± 0.012	0.11	0.019 ± 0.012	0.12
Non-tumor						
Moderate (4–10 ng/mL)	0.010 ± 0.008	0.22	0.016 ± 0.015	0.29	0.010 ± 0.016	0.55
High (>10 ng/mL)	0.009 ± 0.011	0.40	0.019 ± 0.020	0.35	0.023 ± 0.021	0.26
High tumor volume (≥20%)						
Tumor	−0.014 ± 0.004	0.002	−0.012 ± 0.005	0.03	0.004 ± 0.007	0.57
Non-tumor	−0.021 ± 0.007	0.003	−0.011 ± 0.009	0.24	0.014 ± 0.011	0.20
High Prostate Volume (≥65 cc)						
Tumor	−0.005 ± 0.004	0.22	−0.018 ± 0.005	0.001	−0.008 ± 0.007	0.25
Non-tumor	−0.014 ± 0.007	0.04	−0.009 ± 0.009	0.33	0.007 ± 0.011	0.54
Advanced tumor stage (3 or 4)						
Tumor	0.004 ± 0.005	0.51	−0.012 ± 0.007	0.11	−0.004 ± 0.008	0.61
Non-tumor	0.0008 ± 0.009	0.93	−0.001 ± 0.012	0.94	0.013 ± 0.013	0.30
Advanced Gleason Grade[2]						
Tumor	0.003 ± 0.005	0.57	0.013 ± 0.007	0.06	0.010 ± 0.008	0.19
Non-tumor	0.003 ± 0.008	0.70	0.029 ± 0.012	0.02	0.029 ± 0.013	0.02

[1] All beta estimates adjusted for the other five clinical factors.

[2] Gleason sum 8 or higher or Gleason sum 7 and primary Gleason grade 4 or higher.

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SRD5A2 and *HSD3B2* Polymorphisms are Associated With Prostate Cancer Risk and Aggressiveness

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Abstract

BACKGROUND—Dihydrotestosterone (DHT) is believed to play an important role in prostate carcinogenesis. Five alpha reductase type II (*SRD5A2*) and 3 beta-hydroxysteroid dehydrogenase type II (*HSD3B2*) are responsible for the biosynthesis and degradation of DHT in the prostate. Two polymorphisms, a valine (V) for leucine (L) substitution at the 89 codon of the *SRD5A2* gene and a (TG)n,(TA)n,(CA)n repeat polymorphism within the third intron of the *HSD3B2* gene were evaluated with regard to prostate cancer risk.

METHODS—Blood samples were collected for 637 prostate cancer cases and 244 age and race frequency matched controls. In analysis, the *SRD5A2* VL and LL genotypes were combined into one group and the *HSD3B2* repeat polymorphism was dichotomized into short (<283) and long (≥283) alleles.

RESULTS—The *SRD5A2* V89L polymorphism was not independently associated with prostate cancer risk. Carriage of at least one *HSD3B2* intron 3 intron 3 short allele was associated with a significant increased risk for prostate cancer among all subjects (OR = 2.07, 95% CI =1.08–3.95, *P* = 0.03) and Caucasians (OR = 2.80, CI = 2.80–7.43, *P* = 0.04), but not in African Americans (OR = 1.50, CI =0.62–3.60, *P* =0.37). Stratified analyses revealed that most of the prostate cancer risk associated with the intron 3 *HSD3B2* short allele was confined to the *SRD5A2* 89L variant subgroup and indicated that in combination these polymorphisms may be associated with increased risk of aggressive (Gleason >7) disease (Gleason >7).

CONCLUSIONS—In Caucasians, the *HSD3B2* (TG)n,(TA)n,(CA)n intron 3 length polymorphism is associated with both prostate cancer risk and aggressiveness and the *SRD5A2* V89L polymorphism may modify the risk conferred by this polymorphism.

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Keywords

SRD5A2 V89L; *HSD3B2*; prostate cancer; African American

INTRODUCTION

Prostate cancer incidence in African-American men is on average 60% higher than the incidence observed in Caucasian men and mortality from the disease is approximately 2.4 times higher in African-American men [1]. The androgen biosynthesis pathway has been implicated in prostate carcinogens is and frequency differences in gene polymorphisms within this pathway may explain observed race disparities in prostate cancer [2,3]. Androgens are primarily produced in the testes and adrenal glands, but are also synthesized in the prostate and skin. Within the prostate, dihydrotestosterone (DHT) is the primary and most potent nuclear androgen. DHT promotes DNA synthesis and cell replication by binding to the intracellular androgen receptor and forming a complex which activates gene transcription and cell proliferation. Increased cell division is presumed to heighten the potential for somatic mutations, leading to a higher likelihood of carcinogenesis [4]. Two enzymes involved in the regulation of DHT are 5-alpha reductase type II (SRD5A2) and 3 beta-hydroxysteroid dehydrogenase type II (HSD3B2). These enzymes are responsible for the biosynthesis and degradation of DHT.

The 5 α-reductase gene (*SRD5A2*), located on chromosome 2p23 [5], is involved in the conversion of testosterone to DHT in the prostate. SRD5A2 is perhaps best known as the target for finasteride, a drug used to treat benign prostatic hypertrophy and which has shown potential for prostate cancer prevention [6,7]. Several polymorphisms within *SRD5A2* have been identified, including a leucine for valine substitution at codon 89 (*V89L*) [7]. Findings overall have been equivocal in regard to this polymorphism in terms of prostate cancer risk. A meta-analysis of the *SRD5A2 V89L* polymorphism concluded that there was no increased risk associated with prostate cancer (Odds ratio (OR) for L vs. V: 1.03, 95% CI: 0.84–1.26) [8]. However, in vivo and in vitro studies have consistently shown reduced 5 α-reductase activity with the substitution of leucine [9-11] and studies published since the meta-analysis in 2003 have found that the *SRD5A2 V89L* is associated with prostate volume [12] and prostate cancer risk and aggressiveness [13]. Race/ethnicity differences in the frequency of the L variant have also been reported [14].

HSD3B2 functions upstream as well as downstream of SRD5A2 in the androgen pathway and the coding region for the *HSD3B2* gene is located on chromosome 1p13 [15], a region that has shown evidence for linkage to prostate cancer [16-19]. HSD3B2 is one of two enzymes responsible for the degradation of DHT to 3 β-androstanediol [20]. In addition, HSD3B2 is involved in the production of testosterone (T) via its role in converting DHEA to androstenedione, a precursor to testosterone. A complex (TG)n(TA)n(CA)n repeat polymorphism within the third intron of the *HSD3B2* gene has been identified [21], and allelic frequency differences have been observed between African Americans and Caucasians [22]. It has been suggested that the repeat complex may influence the formation of hairpin-like structures that could modify the rate of *HSD3B2* transcription [23] or may promote alternative spliced forms of mRNA resulting in truncated or unstable proteins [24]. Altering intracellular levels of HSD3B2 enzyme could potentially change the rate at which testosterone is produced and/or DHT is degraded. Higher ratios of serum testosterone to DHT have been associated more consistently with prostate cancer risk than either steroid alone [25-27]. To the best of our knowledge no previous reports have assessed the intron 3 repeat *HSD3B2* polymorphism in regard to prostate cancer risk or aggressiveness.

Therefore, in this study, we evaluated the potential associations between the *SRD5A2 V89L* and *HSD3B2* (TG)n(TA)n(CA)n repeat polymorphisms on prostate cancer risk and aggressiveness by race/ethnicity. We also evaluated these polymorphisms in combination since altered activity or expression of both may ultimately have a stronger impact on the availability of DHT and/or testosterone in the prostate potentially leading to increased cell division and prostate carcinogenesis.

METHODS

Study Population and Data Collection

The study population included men that received their primary health care at the Henry Ford Health System (HFHS), a large, vertically integrated, health system. HFHS provides care for an ethnically diverse population that is representative of the geographic region it serves. All study procedures and processes were approved by the HFHS institutional review board. Cases were identified through the centralized Department of Pathology and had histological confirmation of adenocarcinoma of the prostate between January 1999 and December 2004 with no prior history of prostate cancer. The primary objective of the study was to evaluate gene-environment interactions using a case-only analytic approach [28], therefore, the study sampling frame was focused on cases, but controls were also sampled in a stratified random manner from the health system's electronic data stores based on 5-year age group and race, such that the final enrolled sample would include approximately 3 cases per 1 control. Cases and controls were also required to be 75 years of age or younger at diagnosis/enrolment and have at least one primary care contact in the preceding 5 years. The younger age criterion was used in this gene-environment study to enrich the potential genetic contribution to disease among our subjects.

A study introduction letter was sent to all potential subjects and trained study interviewers made initial contact with patients via telephone. Between July 2001 and December 2004, 77% of potential cases and 68% of potential controls enrolled in the study (total N = 881) and provided a blood sample for DNA analysis. Blood samples for controls were also used for prostate specific antigen testing (PSA) at the time of enrolment. Family history of prostate cancer was assessed as part of the patient interview and was considered positive if either a brother or father had been diagnosed with prostate cancer. Cases' cancer stage and Gleason grade were abstracted from the medical record and were verified using the institutions certified tumor registry.

Genotyping

Genotyping of the *SRD5A2 V89L* polymorphism was performed using the Invader assay (biplex format). Each plate contained the following controls: valine/valine homozygous, valine/leucine heterozygous, leucine/leucine homozygous and a no target blank. All components of the assay were provided by Third Wave Technologies, Inc. Ten microliters of genomic DNA samples were aliquoted into individual wells of a 96-well microtitre plate and denatured at 95°C for 5 min. Ten microliters of a reaction mix containing the appropriate probes/Invader oligo/ $MgCl_2$ mix were added, and reactions were overlaid with 20 μl of mineral oil. Each 20 μl reaction contained 40 ng of Cleavase enzyme, 3.5% PEG 8000, 2% glycerol, 0.06% NP40, 0.06% Tween-20, 12 μg/ml BSA, 0.25 μmol/L each of F (FAM) dye and R (Redmond Red) dye FRET cassettes, 8 mmol/L $MgCl_2$, 0.5 μmol/L of each allele-specific probe, and 0.05 μmol/L Invader oligo. The sequences of the oligos and probes are available upon request. The plates were incubated at 63°C for 4 hr in a PE 9700 thermal cycler. Fluorescence was measured using a CytoFluor 4000 fluorescence plate reader (Applied Biosystems, Foster City, CA). The settings used were: 485/20 nm excitation/bandwidth and 530/25 nm emission/bandwidth for F dye detection, and 560/20 nm excitation/bandwidth and 620/40 nm emission/bandwidth for

R dye detection. Any sample that yielded inconclusive results using the Invader assay was re-quantified and repeated. If the second attempt did not yield acceptable results, the sample was genotyped using a PCR-RFLP assay previously described [29].

The *HSD3B2* intron 3 dinucleotide repeat was genotyped by first amplifying genomic DNA using a primer pair previously described [30]. The forward primer was fluorescent tagged with 6-carboxyfluorescerin (FAM). Amplification was performed in a 20 μl final volume containing 200 μM of dCTP, dATP, dGTP, and dTTP, 2 mM $MgCl_2$, 20 pmol each primer, 2.5 U Taq Gold DNA polymerase (Perkin–Elmer), and 1 × Taq Gold buffer. After an initial denaturation step at 95°C for 10 min, 30 cycles of 92°C 2 min, 65°C 1 min and 72°C 2 min were followed by a 45 min final extension at 72°C.

The final products were analyzed by electrophoresis on a 3100 Genetic Analyzer (Applied Biosystems) using GeneScan and Genotyper software. The size of the PCR products for each specimen was determined by the size of the predominant PCR product(s) according to peak area, in relation to the GeneScan-500 ROX size standard (Applied Biosystems). GeneScan results for a sample of homozygous subjects were checked against the actual size of the PCR product as determined through sequence analysis and were determined to be similar.

Statistical Analyses

Hardy–Weinberg equilibrium of genotypic frequencies for both polymorphisms was verified in controls using chi-squared tests. For the multi-allele *HSD3B2* polymorphism, an empirical *P* value was estimated using a simplified Monte Carlo significance test [31]. Case-control differences in categorical variables were assessed using chi-squared tests as well. *SRD5A2 89* valine/leucine (*VL*) and leucine/leucine (*LL*) genotypes were combined for stratified analyses due to low frequencies of the *LL* genotype. Polynomial regression was used to test the linear association of the *HSD3B2* (TG)n(TA)n(CA)n allele lengths with prostate cancer risk. Family history and benign prostatic hyperplasia (BPH) were coded as present or absent and were included in multivariable regression models as potential confounders because of previous reports indicating potential associations with the *SRD5A2 V89L* polymorphism [12, 13,32,33]. Body mass index (continuous) and smoking status (ever/never) were also included in models as potential confounders since each has been shown to influence circulating androgen levels [34]. Age (continuous) was also included in each model and race was adjusted for in analyses of all subjects combined. Aggressive prostate cancer was defined as having Gleason grade of 7–10. Statistical analysis was performed using SPSS (version 11.5). Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between genotypes and prostate cancer risk and aggressiveness, respectively. An alpha level of $P < 0.05$ was considered significant.

RESULTS

Demographics and Clinical Characterization

A total of 637 cases and 244 controls met all criteria and were enrolled in the study (Table I). By design, cases and controls did not differ significantly by race or age. African-American (AA) men represented 43.2% of all participants and were slightly younger than Caucasian (W) subjects (61.8 vs. 62.6 years, $P = 0.06$). Family history of prostate cancer (21.0% vs. 13.1%, $P = 0.01$) and BPH (32.3% vs. 19.7%, $P < 0.0001$) were more prevalent in cases than in controls but did not differ by race (family history: AA 19.4% vs. W 18.6%, $P = 0.76$; BPH: AA 26.8% vs. W 30.6%, $P = 0.34$). Among cases, 55% had a Gleason grade of seven or greater and one in five cases undergoing definitive surgical treatment had a pathological tumor stage of T3 or higher. Gleason grade (AA 57.1% vs. W 53.5%, $P = 0.37$) and pathological tumor stage (AA

18.1% vs. W 20.1%, $P = 0.61$) were not significantly different between African-American and Caucasian cases.

SRD5A2.V *89* LPolymorphism

Among all study subjects, the *SRD5A2 VV* genotype was most common (48.4%), followed by the *VL* (42.2%), and *LL* (9.4%) genotypes. The overall difference in the frequency of the *L* allele by race approached significance (AA 48.3% vs. W 54.3%, $P = 0.08$) and African-American men were significantly less likely to carry the *LL* genotype as compared to Caucasian men (6.8% vs. 11.5%, $P = 0.02$). Among controls, the *SRD5A2 V89L* polymorphism was in Hardy–Weinberg equilibrium. Overall and by race, as Table II indicates, there were no statistically important differences in risk of prostate cancer between cases and controls for the *SRD5A2 V89L* genotype alone.

HSD3B2 Length Polymorphism

A total of 48 *HSD3B2* alleles ranging in length from 213 to 375 were genotyped in our study subjects. Genotype frequencies in controls were in Hardy–Weinberg equilibrium. The 283 bp length allele accounted for 35.3% of all alleles typed. Alleles 286 bp (20.8%) and 333 bp (16.0%) were the next most common alleles with all other alleles occurring at frequencies of less than 10%. Stratification by race showed that in African American and Caucasian controls, the most common alleles occurred at very different frequencies (*HSD3B2* 283 bp: African American 12.4% vs. Caucasian 52.6%; *HSD3B2* 286 bp: African American 36.7% vs. Caucasian 9.6%, *HSD3B2* 333 bp: 11.0% vs. Caucasian 19.9%; Fig. 1). Polynomial regression in African Americans and Caucasians as well as in combined analyses indicated that the relationship between this polymorphism and outcome was not linear. A sensitivity analysis at various binary cut points showed that dichotomizing *HSD3B2* allele lengths into long and short risk alleles at the 283 bp cut point resulted in the formation of risk groups that best differentiated cases from controls. Therefore, we chose to simplify the analysis of this polymorphism by subdividing alleles into two groups of "short" (<283 bp) and "long" (≥283 bp) alleles.

Race-stratified and overall results for the *HSD3B2* polymorphism are shown in Table II. Using the long/long genotype as the referent, the genotypes including short alleles (long/short and short/short) were associated with an elevated risk for prostate cancer in Caucasians (OR 2.80, CI 1.05–7.43, $P = 0.04$) and among all subjects (OR 2.07, CI 1.08–3.95, $P = 0.03$). Risk was also elevated but not significant in African Americans carrying at least one *HSD3B2* (TG)n, (TA)n,(CA)n short allele (OR 1.50, CI .62–3.6, $P = 0.37$).

SRD5A2 and HSD3B2:Prostate Cancer Risk and Aggressiveness

Table III shows the risk of prostate cancer associated with the *HSD3B2* polymorphism after stratification by *SRD5A2 V89L* status. Elevated risk for prostate cancer associated with the long/short or short/short *HSD3B2* genotype were observed for both *SRD5A2* genotypic strata, but within the stratum defined by individuals with either the *SRD5A2 VL or LL* genotypes prostate cancer risk associated with the long/short or short/short *HSD3B2* genotype in Caucasians was elevated (Table II). Risk estimates for Caucasians associated with the *HSD3B2* polymorphism were greater than African Americans even after adjusting for age, family history of prostate cancer, BPH, BMI, and smoking.

We also dichotomized cases based on aggressive disease (Gleason sum ≥7) to assess whether clinical heterogeneity among cases revealed any additional genetic risk (Table III). ORs were significantly increased for aggressive disease within the subset of Caucasians with the *SRD5A2 89L* allele (OR 10.2, CI 1.29–80.5, $P = 0.03$) and among all subjects (OR 3.82, CI 1.27–11.50, $P = 0.02$) even after adjustment for covariates. Overall, stratifying by either the *SRD5A2*

V89L polymorphism or aggressive disease did not appear to affect prostate cancer risk estimates for the *HSD3B2* intron 3 short allele in African Americans.

DISCUSSION

This study sought to elucidate potential independent and joint effects of two polymorphisms in the androgen metabolism pathway, *SRD5A2 V89L* and a (TG)n,(TA)n,(CA)n repeat polymorphism in the third intron of the *HSD3B2* gene, with regard to prostate cancer risk and aggressiveness. Our findings suggest that both of these polymorphisms differ in genotype frequency by race, and that the *HSD3B2* length polymorphism independently and in conjunction with the *SRD5A2 V89L* polymorphism is associated with elevated prostate cancer risk and potentially prostate cancer aggressiveness in all subjects and among Caucasians. The latter result, in particular, must be viewed cautiously since this *HSD3B2* polymorphism has not been previously reported in terms of prostate cancer risk and subject numbers upon which this result was based were very small.

Lachance et al. [21] first reported the *HSD3B2* intron 3 (TG)n,(TA)n,(CA)n repeat and noted hairpin-like structures associated with the polymorphism. These secondary structures have the potential to vary or terminate the rate of transcription of the enzyme [23] or potentially cause alternative spliced forms of mRNA resulting in truncated or unstable proteins [24]. In fact, three alternative spliced forms of the transcribed pre-mRNA of *HSD3B2* are known to exist (http://www.ebi.ac.uk/asd/), with intron 3 excluded from the two shorter transcribed mRNAs that also contain different coding sequences that might alter *HSD3B2* activity and therefore may degrade DHT at varying rates. In terms of which alleles are more likely to form hairpins, previous studies have shown that longer allele length is associated with hairpin structures that are more stable, but this stability is achieved at a certain maximum length such that alleles longer than this threshold offer no further increased stability for the hairpin structure [35,36]. This would support the conservative decision we made to divide the *HSD3B2* length polymorphism into “long” and “short” categories. In addition alternative splicing of pre-mRNA increases genetic diversity [37] and therefore it follows that an allele length that is both long enough to promote alternative spliced forms of the *HSD3B2* protein while at the same time short enough to ensure sufficient copies of the full functional version of the protein are made may be highly selected for.

In our subjects, we found that the short allele (<283 bp) of the *HSD3B2* intron 3 polymorphism was associated with increased risk of prostate cancer and potentially aggressiveness of disease. Carriage of *HSD3B2* short alleles may predispose toward having more copies of the full length HSD3B2 protein that is more active, indirectly producing larger quantities of testosterone and degrading DHT more rapidly resulting in an imbalance of serum testosterone to DHT. Higher serum testosterone to DHT ratios have been associated with increased risk of prostate cancer in some studies [25-27]. But in primary prostate cancer cells DHT levels have been reported to be higher in tumor than adjacent normal cells [38]. If the *HSD3B2* (TG)n,(TA)n,(CA)n polymorphism does have an impact on gene expression, the resulting impact on testosterone and DHT levels may or may not be equivalent. First, DHT is metabolized through multiple pathways most of which are reversible reactions, including the conversion of DHT to 3 β-androstanediol by HSD3B2. It is possible that over expression of *HSD3B2* could have a greater effect on the rate of anabolism of DHT than the rate of catabolism of DHT. Secondly, Ji et al. [38] have reported on two proteins also involved in the metabolism of DHT, AKR1C1 and AKR1C2. Although AKR1C1, which is associated with the HSD3B pathway of DHT metabolism, is expressed at higher levels than AKR1C2, AKR1C2 had more influence on DHT-dependent androgen receptor reporter activity. In prostate cancer, the HSD3B2 metabolic pathway of DHT, therefore, may be out competed by other metabolic pathways. The higher expression yet lower affect of the AK1C1 protein would suggest this.

In vivo and in vitro studies of the *SRD5A2 V89L* polymorphism have indicated reduced activity of the SRD5A2 enzyme with the leucine substitution [9-11] indicating a slower conversion rate of testosterone to DHT. In terms of prostate cancer risk, this polymorphism has shown mixed results. A meta analysis that included the review of 12 studies was conducted by Ntais et al. [8] and indicated no overall change in risk with the *L* variant. Since this analysis Forrest et al. [39] in a case-control analysis using controls from the EPIC study reported increased risk for prostate cancer associated with the *LL* genotype and Cicek et al. [13] found increased risk was primarily driven by men diagnosed at younger age or with more aggressive disease. This polymorphism has also been associated with progression of disease [40]. Stanbrough et al. [41] found increased levels of HSD3B2 activity and decreased levels of SRD5A2 (approximately 50%) in metastatic androgen-independent prostate cancer tumors. Others have also shown lower levels of SRD5A2 in prostate tissue [42-44]. The increased prostate cancer risk we found associated with Caucasian carriers of the *HSD3B2* intron 3 short allele and *SRD5A2 89L* allele that may predispose to higher levels of HSD3B2 but less active SRD5A2 enzyme are in line with these findings. Among African Americans, risk associated with the *HSD3B2* intron 3 short allele was elevated but not significant and stratification by the *SRD5A2 V89L* polymorphism appeared not to impact risk. Interestingly, the most common *HSD3B2* intron 3 repeat allele length was different in Caucasians (283 bp) and African Americans (286 bp) suggesting that some evolutionary advantage selecting for the longer allele length in African populations may exist. In addition, the frequency of the LL genotype in our African-American sample was at the lower end of previously reported ranges [13,14,45] limiting the statistical power for testing associations of this polymorphism. The V89L polymorphism in exon 1 of the *SRD5A2* gene lies at the far 3′ end of the gene and therefore is in linkage equilibrium with several other polymorphisms within the *SRD5A2* gene that have been described by Reichardt et al. [46] and others [47,48]. In terms of prostate cancer risk, Loukola et al. [47] interrogated the entire *SRD5A2* gene and found 25 SNPs, but only the V89L variant and another SNP in an untranslated region of the gene in strong linkage disequilibrium with the V89L variant were associated with prostate cancer. Polymorphism in *HSD3B2* may be limited, with a recent sequencing study [49] finding only six SNPs. The few coding SNPs in *HSD3B2* that have been reported are not polymorphic enough to be of any practical utility for testing associations with disease risk in moderately sized samples. Interestingly, while the *HSD3B2* intron 3 repeat polymorphism was reported to vary significantly with race 10 years ago [22], until the current study it has never been investigated in the context of prostate cancer risk.

Although our study sample size was relatively large and included a large proportion of African-American subjects, the original study design included only one age-race frequency matched control to every three cases limiting statistical power for stratified case-control comparisons. The controls were representative of the population from which the cases were sampled and controlling for family history and BPH, two factors which differed by case-control status, showed no important difference from crude odds ratios. We also compared mean rank PSA levels for all genotypes and haplotypes (Tables II and III) using the Kruskal–Wallace test, as genetic variations in genes that control androgens may affect PSA levels and detection of disease. We found no significant differences in PSA levels between groups, reducing the possibility that detection bias lead to our observations. In addition, the frequency of high Gleason scores in our subjects may have contributed to our findings on aggressive disease, as nearly 65% of our case subjects underwent prostatectomy. Gleason scores have been shown to increase from biopsy in prostatectomy cases [50] and the higher use of radical prostatectomy at our institution may have provided higher than expected frequencies of aggressive cases. None of the gene–gene interactions we tested were statistically significant, nonetheless interaction odds ratios in Caucasians were greatly elevated suggesting a possible synergy of effect between *HSD3B2* and *SRD5A2* in the prostate carcinogens is pathway.

To our knowledge, our study represents the first report of an association between the *HSD3B2* (TG)n,(TA)n,(CA)n polymorphism with risk of prostate cancer. Risk and aggressiveness of disease among all subjects and in Caucasians was associated with short allele genotypes of the *HSD3B2* (TG)n,(TA)n,(CA)n and the *SRD5A2 V89L* polymorphism may modify this risk. Further study of this repeat polymorphism, independently and in conjunction with other genetic polymorphism within the androgen pathway is clearly warranted.

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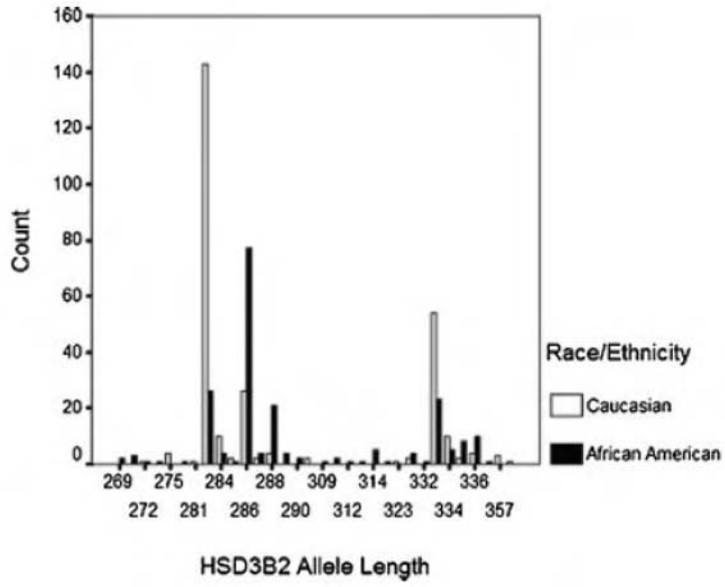

Fig. 1.
HSD3B2 repeat polymorphism allele distribution among controls by race.

TABLE I

Patient Characteristics

Characteristic	Controls N =244 n (%)	Cases N =637 n (%)	*P*-value*
Race			
African American	104 (42.7)	274 (43.0)	0.92
White	140 (57.4)	363 (57.0)	
Age			
<60	76 (31.2)	228 (35.8)	0.39
60–69	130 (53.3)	323 (50.7)	
70+	38 (15.6)	86 (13.5)	
Smoking			
Never	82 (33.6)	219 (34.4)	0.83
Ever	162 (66.4)	418 (65.6)	
BMI			
<25	45 (18.4)	127 (19.9)	0.43
25–29	108 (44.3)	302 (47.4)	
≥30	91 (37.3)	208 (32.7)	
Family history			
Positive	32 (13.1)	134 (21.0)	0.01
Negative	212 (86.9)	503 (79.0)	
BPH history			
Positive	48 (19.7)	206 (32.3)	<0.0001
Negative	193 (79.1)	430 (67.5)	
Unknown	3 (1.2)	1 (0.2)	
PSA level			
<4	223 (91.4)	108 (17.0)	<0.0001
4–10	17 (7.0)	424 (66.6)	
>10	4 (1.6)	105 (16.5)	
Path tumor stage[a]			
<T3		339 (79.8)	
≥T3		86 (20.2)	
Gleason 4–6	—	279 (43.9)	
7		254 (39.9)	
8–10		97 (15.3)	

[a] Surgical cases only.

* *P*-value significant at <0.05.

TABLE II

Association Between *SRD5A2* and *HSD3B2* Genotype Frequencies and Risk of Prostate Cancer

***SRD5A2* genotype**	**Controls n (%)**	**Cases n (%)**	**OR (CI)[a]**	***P*-value[*]**
African Americans				
VV	54 (50.9)	143 (52.0)	Reference	
VL	45 (42.5)	113 (41.1)	0.89 (0.55–1.44)	0.64
LL	7 (6.6)	19 (6.9)	0.99 (0.62–1.59)	0.97
Total	106 (100.0)	275 (100.0)		
Caucasians				
VV	66 (48.5)	160 (44.7)	Reference	
VL	53 (39.0)	158 (44.1)	1.33 (0.86–2.06)	0.20
LL	17 (12.5)	40 (11.2)	1.01 (0.73–1.40)	0.96
Total	136 (100.0)	358 (100.0)		
All subjects				
VV	120 (49.6)	303 (47.9)	Reference	
VL	98 (40.5)	271 (42.8)	1.11 (0.81–1.53)	0.51
LL	24 (9.9)	59 (9.3)	0.98 (0.76–1.28)	0.92
Total	242 (100.0)	633 (100.0)		
HSD3B2 genotype	Controls n (%)	Cases n (%)	OR (CI)[a]	*P*-value[*]
African Americans				
Long/long	98 (93.3)	247 (90.1)	Reference	
Long/short or short/short	7 (6.7)	27 (9.9)	1.50 (0.62–3.6)	0.37
Total[b]	105 (100.0)	274 (100.0)		
Caucasians				
Long/long	131 (96.3)	325 (90.8)	Reference	
Long/short or short/short	5 (3.7)	33 (9.2)	2.80 (1.05–7.43)	0.04
Total	136 (100.0)	358 (100.0)		
All subjects				
Long/long	229 (95.0)	572 (90.5)	Reference	
Long/short or short/short	12 (5.0)	60 (9.5)	2.07 (1.08–3.95)	0.03
Total	241 (100%)	632 (100%)		

[a] Odds Ratios adjusted for race (all subjects), age, family history, BPH, BMI and smoking history, CI 95%.

[b] After multiple attempts, two African Americans could not be genotyped for the *HSD3B2* polymorphism.

[*] *P* value significant at <0.05.

TABLE III

Association Between *SRD5A2* and *HSD3B2* Haplotype and Prostate Cancer and Aggressiveness

***SRD5A2* genotype**	***HSD3B2* genotype**	**Controls n (%)**	**All cases n (%)**	**OR (CI)[a]**	***P*-value[**]**	**Case Gleason 4–6[a] OR (CI)**	***P*-value[**]**	**Case Gleason 8–10[a] OR (CI)**	***P*-value[**]**
VV	African Americans								
	Long/long	49 (92.5)	129 (90.8)	Reference		Reference		Reference	
	Long/short or short/short	4 (7.5)	13 (9.2)	1.62 (0.48–5.48)	0.43	1.99 (0.46–8.65)	0.36	1.50 (0.41–5.44)	0.54
	Caucasians								
	Long/long	62 (93.9)	146 (91.3)	Reference		Reference		Reference	
	Long/short or short/short	4 (6.1)	14 (8.8)	1.66 (0.50–5.44)	0.40	1.49 (0.38–5.74)	0.56	1.86 (0.49–7.02)	0.36
	All subjects								
	Long/long	111 (93.3)	275 (91.1)	Reference		Reference		Reference	
	Long/short or short/short	8 (6.7)	27 (8.9)	1.62 (0.70–3.76)	0.26	1.62 (0.62–4.27)	0.33	1.65 (0.66–4.15)	0.29
VL or LL	African Americans								
	Long/long	49 (94.2)	118 (89.4)	Reference		Reference		Reference	
	Long/short or short/short	3 (5.8)	14 (10.6)	1.64 (0.42–6.33)	0.48	2.05 (0.43–9.71)	0.37	1.57 (0.36–6.77)	0.55
	Caucasians								
	Long/long	69 (98.6)	179 (90.4)	Reference		Reference		Reference	
	Long/short or short/short	1 (1.4)	19 (9.6)	7.42 (0.96–57.4)	0.06	4.37 (0.48–39.7)	0.19	10.18 (1.29–80.48)	0.03
	All subjects								
	Long/long	118 (96.7)	297 (90.0)	Reference		Reference		Reference	
	Long/short or short/short	4 (3.3)	33 (10.0)	3.16 (1.08–9.25)	0.04	2.60 (0.78–8.69)	0.12	3.82 (1.27–11.50)	0.02

[a] Odds ratios adjusted for race (all subjects), age, family history, BPH, BMI, smoking, 95% CI.

[**] *P*-value significant at <0.05.

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Polycyclic Aromatic Hydrocarbon – DNA Adducts in Prostate and Biochemical Recurrence after Prostatectomy

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Abstract

Purpose: DNA adduct levels may be influenced by metabolic activity, DNA repair capabilities, and genomic integrity, all of which play a role in cancer progression.

Experimental Design: To determine if elevated DNA adducts are a marker for prostate cancer progression, we measured polycyclic aromatic hydrocarbon – DNA adducts by immunohistochemistry in prostate cells of 368 surgical prostate cancer patients treated at the Henry Ford Hospital in Detroit, Michigan, between September 1999 and July 2004. Patients were followed up to 5 years after surgery with relative risk for biochemical recurrence (BCR) estimated with a Cox proportional hazards model that adjusted for standard clinical risk factors.

Results: At 1 year of follow-up, patients with adduct levels above the median in tumor cells [hazard ratio (HR), 2.40; 95% confidence interval (95% CI), 1.10-5.27] and nontumor cells (HR, 3.22; 95% CI, 1.40-7.39) had significant increased risk of BCR, but these HRs decreased to 1.12 (95% CI, 0.68-1.83) and 1.46 (95% CI, 0.89-2.41) in tumor and nontumor cells at 5 years postsurgery. When we restricted our analysis to patients with advanced-stage (III+) disease, those with high adduct levels in either tumor (53.5% versus 30.2%; $P = 0.07$) or nontumor (55.2% versus 28.6%; $P = 0.02$) cells had BCR rates almost 2-fold higher. In race-stratified analyses, the greatest risk of BCR associated with high adduct levels (in nontumor cells) was for African American patients younger than 60 years old (HR, 3.79; 95% CI, 1.01-14.30).

Conclusions: High polycyclic aromatic hydrocarbon – DNA adduct levels in nontumor prostate cells are most strongly associated with BCR between 1 and 2 years after surgery and in patient subsets defined by younger age, advanced tumor stage, and African American race.

Polycyclic aromatic hydrocarbons (PAH) result from incomplete combustion processes, are ubiquitous environmental contaminants, and are known carcinogens (1). PAH derive their carcinogenic properties through their ability to form PAH-DNA adducts (2, 3). *In vitro* experiments have detected DNA adducts in human prostate after exposure to benzo(*a*)pyrene (4, 5), a known carcinogenic PAH, and have shown that exposure levels of benzo(*a*)pyrene are positively correlated with DNA damage as measured by the comet assay (6). In men diagnosed with prostate cancer who underwent radical prostatectomy, we found that levels of PAH-DNA adducts in prostate epithelial cells were inversely related to tumor grade (7), and more recently have also shown that the effects of underlying genetic variation in PAH-metabolizing enzymes and cigarette smoke exposure leading to PAH-DNA adduct formation in the prostate may be different by race (8).

Retrospective epidemiologic studies support a link between occupational PAH exposure and prostate cancer risk (9–11), but a recent prospective cohort study was unable to replicate this association (12). Occupational PAH exposure may need to reach a threshold level before having an effect on cancer risk (13, 14), and genetic susceptibility likely also plays a role (13, 15). Other environmental sources of PAH include diet (16) and cigarette smoke (17). Whether an increased prostate cancer risk is associated with cigarette smoke is unclear (18–20), although several recent studies suggest that cigarette smoke exposure in combination with genetic risk factors for bulky PAH-DNA adduct formation may increase prostate cancer risk (13, 15, 21). Dietary intake of PAH is primarily through consumption of well-done meats, but epidemiologic evidence for an association between meat consumption and increased risk for prostate cancer is equivocal (22). Only one epidemiologic study has examined dietary intake of benzo(*a*)pyrene, the primary PAH in well-done meats, and prostate cancer risk, but it had null results (23).

Whereas prior examination of PAH exposures on prostate cancer risk has predominantly relied on self-reported measures, PAH-DNA adducts may serve as a marker of the biologically

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effective dose of all types of PAH exposure that is less prone to information bias. For prostate cancer patients, predicting who will have recurrent disease after primary treatment has traditionally relied on clinical and pathologic variables such as Gleason grade and tumor stage. More recently, molecular approaches to predicting prostate cancer recurrence using proteomic and expression array technologies have expanded the potential markers of poor disease outcome (24), but biomarkers currently under investigation lack information about the prostate cell DNA integrity and capacity to metabolize and clear carcinogens. In addition to an individual's PAH exposures, PAH-DNA adduct levels in the prostate reflect metabolic capacity to activate PAH compounds for DNA binding, PAH detoxification capacity, and DNA repair capacity, biological variables that may also be related to cancer prognosis. For instance, the same metabolic enzymes that activate PAH adduct–forming compounds, such as CYP1B1, may also stimulate cancer cell growth and division (25, 26).

If PAH-DNA adducts in prostate cells are indicative of the overall metabolic activity, DNA repair, and genomic integrity of the prostate, then they may be related to prostate cancer progression. To test this hypothesis, we measured PAH-DNA adduct levels in tumor and adjacent nontumor prostate cells of men that had a radical prostatectomy and then followed these men for prostate-specific antigen (PSA) failure to determine whether adduct levels could predict recurrent disease.

Materials and Methods

Study sample ascertainment and follow-up. Between July 1, 2001, and December 31, 2004, we attempted to enroll 863 men that had a prostate cancer diagnosis within the last 2 years at the Henry Ford Health System in Detroit, Michigan, as part of a prostate cancer-case control study (13), and 668 agreed to participate (77%). During the course of enrollment, 8 cases were found ineligible and 23 cases did not complete the study protocol, resulting in final study participation percentages of 75% (637 of 855). Of these 637 cases, 419 (66%) underwent radical prostatectomy. Tissue specimens with sufficient areas of tumor and nontumor cells were available for 392 (94%) of these patients such that immunohistochemical studies for PAH-DNA adduct determination could be done. For these 392 patients, whose dates of prostatectomy occurred between September 1, 1999, and December 27, 2004, we then electronically retrieved all PSA tests from the date of surgery forward. A total of 3,413 test results were retrieved, with the men in this sample having a median of eight PSA tests and the number of tests ranging from 0 to 46 tests. We excluded the 2 men who had no PSA tests, 8 men who had only one PSA test following surgery, and 14 men who also had hormone treatment. The remaining 368 men comprised the analytic study sample. All protocols used in this study were reviewed and approved by the Henry Ford Hospital Institutional Review board and all study participants signed an informed consent before participating.

Pathology. H&E-stained slides of study cases were reviewed by the study pathologist (A.T.S.) to confirm the diagnosis and identify a paraffin block with sufficient tumor and nontumor prostatic tissue for staining. For each patient sample, consecutive sections (5-μm-thick) were cut from the tissue block. One slide was H&E stained and examined by the study pathologist who circled two separate areas of tumor and nontumor cell populations to be used for adduct scoring. Tumors were characterized according to lymph node involvement, primary and secondary grade (i.e., Gleason score), lobe involvement, extraprostatic extension, and seminal vesicle involvement.

Immunohistochemistry. The immunohistochemical assay for PAH-DNA adducts was carried out as described previously (27, 28). This chemical assay uses the monoclonal 5D11 antibody, which in cell culture studies has been shown to produce strongly correlated staining levels ($r = 0.99$; $P = 0.011$) with the treatment dose of benzo(a)pyrene diol epoxide (29, 30). Consistent with our previous study (7) and other prior studies (27, 31) using immunohistochemical assays to measure PAH-DNA adducts, we report our results in absorbance units, which provide a measure of the relative intensity of staining. For each prostate specimen, two technicians independently scored 50 epithelial cells (five fields with 10 cells per field scored) in the two areas (tumor and nontumor) circumscribed by the study pathologist. Scored cells were selected to be representative, in terms of intensity, of the cells in the field and the mean of the two technicians' scores was used. The dual scoring technique has proven to yield a high test-retest reliability in prostate cells (7). PAH-DNA adduct data were standardized across experiments using a series of two "control" slides cut from two separate nonstudy prostate specimens that were run across all batches.

Statistical analyses. A biochemical recurrence (BCR) event was defined as having two consecutive detectable (>0.2 ng/mL) increasing PSA levels 4 weeks or more after surgery (32, 33). Time to event was the duration between the dates of surgery and the second PSA test that defined the recurrence event or censored at the last postoperative PSA test for men that did not recur. HRs for BCR were estimated with Cox proportional hazards models using PROC PHREG in the Statistical Analysis Software package (34). Differences in survival curves were tested using the Wilcoxon rank test in PROC LIFETEST. In addition to adduct levels measured in absorbance units, multivariable models included age, race, pack-years of cigarette smoking, tumor stage, Gleason grade, and preoperative PSA level.

Results

PAH-DNA adduct levels in the prostate tumor and nontumor cells of 368 study participants did not vary significantly by age, race, dietary PAH intake, body size, family history of prostate cancer, PSA at surgery, or advanced tumor grade (Table 1). Tumor stage was significantly inversely associated with PAH-DNA adduct levels in tumor and nontumor cells, and current smokers had a suggestive, albeit nonsignificant, association with higher PAH-DNA adduct levels in both nontumor and tumor cells. A BCR event was experienced by 67 (18.2%) men in the analytic sample that had a median time to recurrence of 14 months with recurrence times ranging between 1.5 and 60 months. Men without a BCR event had follow-up ranging from 2 to 81 months with a median follow-up time of 38 months. For the purposes of analysis and presentation of survival data, all follow-up was censored at 60 months. Men that experienced BCR were more likely to have tumors with advanced Gleason grade, advanced tumor stage, and higher PSA levels at diagnosis (Table 2). Age, race, and smoking status were not associated with BCR nor were mean PAH-DNA adduct levels in either tumor or nontumor prostate cells. Quantifying smoking exposure by pack-years showed that men that experienced prostate cancer recurrence had a marginally higher exposure level to cigarette smoke compared with those that did not recur (24.3 ± 28.2 versus 17.8 ± 22.9 pack-years; $P = 0.08$).

To determine whether PAH-DNA adduct levels were associated with BCR in prostate cancer in a nonlinear fashion, we investigated associations between time to BCR and adduct levels by quartile and median in tumor and nontumor prostate cells. There was no evidence for a trend by quartile in either tumor ($P = 0.78$) or nontumor ($P = 0.26$) cells. In tumor cells, the hazard ratio (HR) associated with PAH-DNA adduct levels above the median was slightly elevated, but not statistically significant [HR, 1.18; 95% confidence interval (95% CI), 0.72-1.94; $P = 0.51$]. In nontumor cells, a larger HR was

Table 1. PAH-DNA adduct levels in tumor and nontumor prostate cells of 368 prostate cancer cases by selected characteristics

Characteristic	Tumor cells	P	Nontumor cells	P
Age				
<60	0.152 ± 0.004	0.31	0.247 ± 0.006	0.92
60+	0.147 ± 0.004		0.246 ± 0.005	
Race				
African American (*n* = 162)	0.150 ± 0.004	0.67	0.246 ± 0.006	0.99
Caucasian or other*(*n* = 206)	0.148 ± 0.004		0.246 ± 0.005	
Cigarette smoking status				
Never (*n* = 136)	0.144 ± 0.005	0.07	0.241 ± 0.007	0.31
Former (*n* = 194)	0.149 ± 0.004		0.246 ± 0.006	
Current (*n* = 38)	0.166 ± 0.009		0.263 ± 0.013	
Dietary PAH intake				
Below median (<; *n* = 184)	0.153 ± 0.004	0.17	0.250 ± 0.006	0.30
Above median (>; *n* = 184)	0.145 ± 0.004		0.242 ± 0.006	
Body size				
Normal (BMI <25 kg/m^2; *n* = 80)	0.145 ± 0.006	0.65	0.235 ± 0.009	0.37
Overweight (BMI 25-29.9 kg/m^2; *n* = 190)	0.151 ± 0.004		0.249 ± 0.006	
Obese (BMI 30+; *n* = 98)	0.148 ± 0.005		0.250 ± 0.008	
Family history †				
Negative (*n* = 269)	0.149 ± 0.003	0.50	0.246 ± 0.005	0.50
Positive (*n* = 90)	0.144 ± 0.005		0.239 ± 0.008	
PSA at surgery (ng/mL)				
<4 (*n* = 65)	0.153 ± 0.007	0.72	0.252 ± 0.010	0.23
4-10 (*n* = 245)	0.147 ± 0.003		0.241 ± 0.005	
>10 (*n* = 58)	0.151 ± 0.007		0.259 ± 0.010	
Pathologic tumor stage				
2 (*n* = 297)	0.153 ± 0.003	0.007	0.252 ± 0.005	0.006
3 or 4 (*n* = 71)	0.134 ± 0.006		0.246 ± 0.005	
Advanced tumor grade ‡				
No (*n* = 259)	0.150 ± 0.003	0.27	0.246 ± 0.005	0.46
Yes (*n* = 109)	0.146 ± 0.005		0.247 ± 0.008	

NOTE: PAH-DNA adduct levels are measured in absorbance units.
Abbreviation: BMI, body mass index.
*"Other" includes one Asian and two Hispanic cases.
† Positive family history is defined as having a brother or father diagnosed with prostate cancer; nine had unknown family history.
‡ Advanced tumor grade is defined as total Gleason grade of 8 or higher or total Gleason grade of 7 and primary Gleason grade of 4 or higher.

observed, but it did not reach statistical significance (HR, 1.56; 95% CI, 0.94-2.59; $P = 0.08$).

The unadjusted BCR distributions stratified according to high and low PAH-DNA adduct levels in tumor (Fig. 1A) and nontumor (Fig. 1B) prostate cells were not significantly different ($P = 0.88$ in tumor cells; $P = 0.11$ in nontumor cells). Although the survival curves tended to move toward each other as follow-up time increased, at earlier follow-up durations

Table 2. Characteristics of 368 prostate cancer cases by BCR status after surgery

Characteristic	No recurrence (*n* = 301)	Recurrence (*n* = 67)	P
Age	61.0 ± 6.8	60.9 ± 6.1	0.84
Percent African American	44.2	43.3	0.89
Observation time (mo)*	51.0 ± 16.1	54.5 ± 17.6	0.13
PSA at diagnosis (ng/mL)	6.0 ± 4.2	11.1 ± 10.1	0.0001
Advanced Gleason grade †	16.9	49.3	<0.0001
Advanced tumor stage (III or IV)	14.3	41.8	<0.0001
Cigarette smoking status			
Never	38.2	31.3	0.56
Former	51.5	58.2	
Current	10.3	10.5	
PAH-DNA adduct level in tumor cells ‡	0.149 ± 0.053	0.149 ± 0.053	0.96
PAH-DNA adduct level in nontumor cells ‡	0.245 ± 0.078	0.252 ± 0.079	0.49

*Time from study entry to date of last PSA test for the entire cohort.
† Gleason sum of 8 or higher or primary Gleason grade 4 or higher.
‡ Expressed in absorbance units.

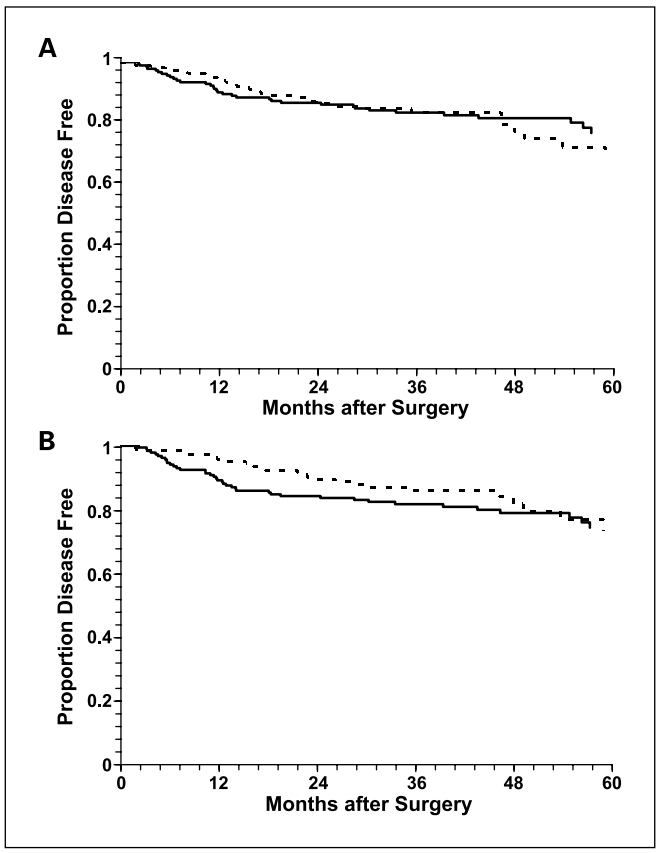

Fig. 1. Kaplan-Meier survival curves for BCR in prostate cancer patients stratified by low (below median, *broken line*) and high (above median, *solid line*) PAH-DNA adduct levels in tumor (*A*) and nontumor (*B*) cells.

(up to 2 years for tumor cells and 4 years in nontumor cells), higher adduct levels were associated with a higher event rate. To quantify the association of high PAH-DNA adduct levels and BCR by follow-up time, we recalculated the HRs for follow-up times ranging from 1 to 3 years (Table 3). In tumor cells, the strongest association with higher PAH-DNA adduct levels was at 1 year of follow-up. In the third and fourth highest quartiles of adduct levels, HRs were both greater than 3. The test for trend for increasing risk across the four quartiles was statistically significant (P = 0.03) and the HR for PAH-DNA adduct levels above the median was 2.41 (95% CI, 1.10-5.29). In nontumor cells, the strongest association with higher PAH-DNA adduct levels was also observed at 1 year of follow-up. In the third and fourth highest quartiles of adduct levels, HRs were 3.83 and 3.45, respectively. The test for trend for increasing risk across the four quartiles was statistically significant (P = 0.01) and the HR for PAH-DNA adduct levels above the median was 3.24 (95% CI, 1.41-7.42). The HRs for PAH-DNA adduct levels above the median in nontumor cells were significant for follow-up periods up to 3 years and were consistently higher than comparable HRs for high PAH-DNA adduct levels in tumor cells.

We next investigated whether high PAH-DNA adduct levels in tumor and nontumor prostate cells might have stronger associations with BCR in patient subsets defined by known clinical risk factors such as high PSA level at diagnosis, advanced tumor stage, and advanced Gleason grade. We also examined patients dichotomized by race (Caucasian, African American) and age (<60, ≥60 years) with age categories based largely on the distribution of patients in the study sample, but also driven by several studies that suggest prostate cancer patients younger than 60 years old have worse outcomes (35–37). Of these five factors, tumor stage was the strongest modifying factor of the association between high PAH-DNA adduct levels in tumor cells and BCR. In patients with tumor stage III or IV, those with high PAH-DNA adducts had almost a 2-fold greater BCR rate over 5 years (53.5% versus 30.2%; P = 0.07). In nontumor cells, age and PSA level as well as tumor stage had differential associations with BCR (Fig. 2A-C). In

Table 3. Risk of BCR after prostatectomy in 368 prostate cancer cases at different lengths of follow-up associated with PAH-DNA adduct levels in prostate cells adjusting for clinical risk factors

Cell type	Follow-up period			
Model variable	**1 y**	**18 mo**	**2 y**	**3 y**
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Tumor adduct level				
2nd Quartile	1.79 (0.49-6.52)	1.06 (0.42-2.67)	0.99 (0.42-2.34)	0.79 (0.36-1.76)
3rd Quartile	3.43 (1.09-10.77)	1.71 (0.75-3.90)	1.54 (0.71-3.34)	1.37 (0.68-2.74)
4th Quartile	3.05 (0.82-11.38)	1.31 (0.47-3.61)	1.39 (0.56-3.46)	1.12 (0.49-2.54)
Linear trend	1.50 (1.04-2.17)	1.17 (0.87-1.57)	1.17 (0.88-1.54)	1.10 (0.86-1.42)
Above median	2.41 (1.10-5.29)	1.52 (0.80-2.87)	1.48 (0.82-2.70)	1.41 (0.82-2.43)
Nontumor adduct level				
2nd Quartile	1.29 (0.31-5.35)	0.96 (0.32-2.84)	0.89 (0.33-2.40)	0.80 (0.34-1.91)
3rd Quartile	3.83 (1.20-12.27)	3.15 (1.32-7.48)	2.66 (1.20-5.91)	1.89 (0.92-3.92)
4th Quartile	3.45 (1.04-11.43)	1.82 (0.69-4.82)	1.77 (0.73-4.27)	1.50 (0.69-3.28)
Linear trend	1.57 (1.11-2.22)	1.34 (1.01-1.78)	1.32 (1.01-1.71)	1.23 (0.96-1.57)
Above median	3.24 (1.41-7.42)	2.56 (1.32-4.99)	2.35 (1.27-4.34)	1.89 (1.09-3.28)
No. events	28	40	46	55

NOTE: Clinical risk factors include PSA, tumor grade, and tumor stage.

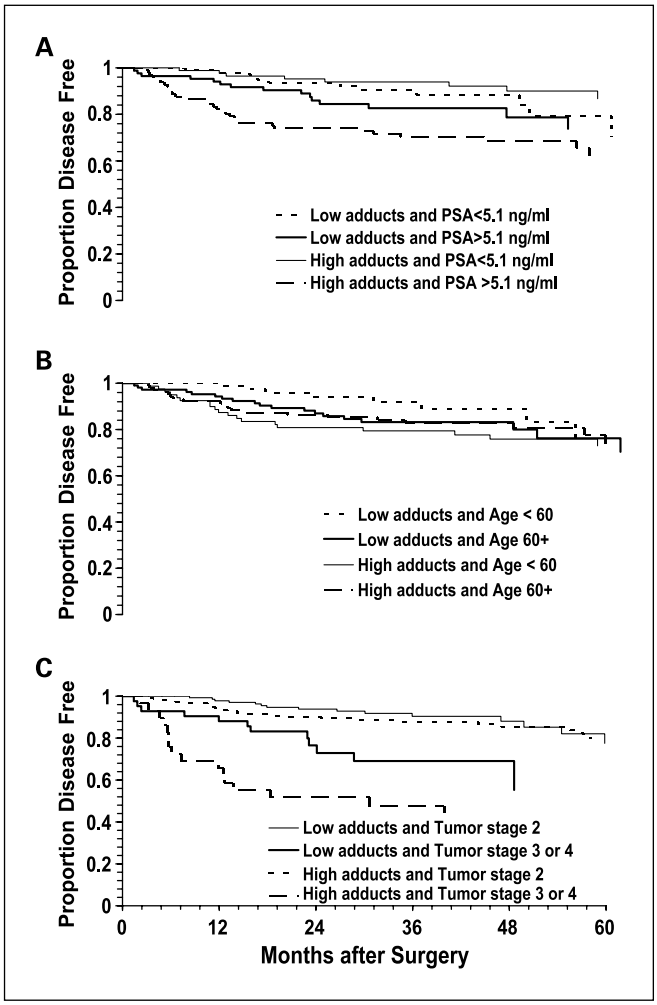

Fig. 2. Kaplan-Meier survival curves for BCR in prostate cancer patients stratified by PAH-DNA adduct levels (above vs below median) in nontumor cells and (*A*) PSA at diagnosis; (*B*) age at diagnosis; and (*C*) tumor stage.

separate analyses of the higher risk groups for these three factors, the BCR rates for high PAH-DNA adduct levels were significantly different in patients with tumor stage III or IV (55.2% versus 28.6%; $P = 0.02$), younger than 60 years old (23.8% versus 10.4%; $P = 0.02$), and with PSA levels above the median (31.6% versus 17.6%; $P = 0.03$).

To determine how BCR associations with high adduct levels varied with follow-up time within patient subsets, we calculated HRs associated with high PAH-DNA adduct levels in tumor and nontumor cells for patient subsets defined by tumor stage, age at surgery, and PSA level at diagnosis at 2, 3, and 5 years of follow-up (Table 4). In tumor cells, the HR for high PAH-DNA adduct levels in patients with advanced tumor stage was consistently elevated in the range of 1.86 to 1.93 between 2 and 5 years of follow-up. In nontumor cells, high PAH-DNA adduct levels were associated with the greatest risk for BCR in patients younger than 60 years old after 2 years of follow-up (HR, 4.62; 95% CI, 1.49-14.35). The elevated risk in the younger age group dissipated as follow-up times were extended, but at 5 years of follow-up high PAH-DNA adduct levels still conferred a risk of BCR greater than 2 (HR, 2.21; 95% CI, 0.94-5.26) in the younger patient group. Patients with PSA levels above the median (≥5.1 ng/mL) had significantly increased risk of BCR associated with high adduct levels across all three follow-up intervals, ranging from a HR of 2.41 at 2 years of follow-up to 1.91 at 5 years of follow-up. For patients with advanced tumor stage (III or IV), the risk of BCR was greatest at 2 years of follow-up (HR, 2.53; 95% CI, 1.07-5.99), and remained elevated through 5 years of follow-up.

In the full sample, high DNA adduct levels had similar associations with BCR in African Americans and Caucasians; however, in the stratified clinical subsets in which high DNA adduct levels had the strongest association with BCR, HRs for African Americans tended to be greater. In tumor cells, elevated DNA adduct levels were associated with higher HRs in younger (2.02 versus 1.26) and advanced-stage (2.15 versus 1.50) African Americans compared with Caucasian patients. In nontumor cells, the highest HR associated with elevated DNA adduct levels was observed for younger African-American patients (HR, 3.79; 95% CI, 1.01-14.30), whereas the comparable HR for Caucasians was only 1.72 (95% CI, 0.51-5.69). Because cigarette smoking can be considered an antecedent variable in the putative adduct-prostate carcinogenesis pathway, it was not included in our multivariable analyses of PAH-DNA adduct levels. However, given the marginal association of cigarette smoking with BCR, and the possibility that this association may not be fully explained by adduct formation, we reran all multivariable models including a covariate for pack-years of smoking. The resulting β estimates for PAH-DNA adduct levels were only nominally (<10%) changed in all circumstances.

Discussion

The current paradigm of DNA adduct formation associates adducts with the initiation phase of carcinogenesis in which an activated xenobiotic compound binds and damages a DNA molecule. In reality, DNA adducts may be relevant to all stages of carcinogenesis as biomarkers of underlying risk related to an individual's ability to both metabolize carcinogens and repair damaged DNA. In the present study, we have shown that PAH-DNA adduct levels in prostate at time of diagnosis may be a biomarker of increased risk for early BCR. We also found that PAH-DNA adducts were inversely associated with tumor stage, but not tumor grade, which is in contrast to our previous reports (7, 8). It should be noted, however, that the eligibility criteria for the present study were different than that of the two previous studies. Further, in the present study, we defined tumor grade and stage in a dichotomous fashion to facilitate survival analyses, which was different than how we defined these variables in our original report (7).

The association between PAH-DNA adducts and BCR risk was greater for adduct levels in nontumor cells, which may better reflect inherited genetic capabilities of xenobiotic metabolism and DNA repair rather than adduct levels in tumor cells where changes in the genetic background have occurred due to somatic mutations. Consistent with what we have previously reported (7, 8), adduct levels were higher in nontumor cells compared with tumor cells. Several studies of other tissues that measured PAH-DNA adducts in both tumor and adjacent nontumor cells, including lung (38), laryngeal (39), pancreas (40), and liver (41), have also reported higher

Table 4. Risk of BCR after prostatectomy associated with high PAH-DNA adduct levels in prostate cells adjusting for clinical risk factors at different lengths of follow-up in selected subsets of prostate cancer cases

Cell type follow-up period	HR (95% CI)	HR (95% CI)	P*
Tumor	Age <60 (n = 157)	Age ≥60 (n = 211)	
2 y	2.54 (0.93-6.92)	0.97 (0.44-2.12)	0.13
3 y	1.75 (0.71-4.32)	1.11 (0.55-2.24)	0.35
5 y	1.46 (0.64-3.33)	0.98 (0.51-1.87)	0.41
Nontumor			
2 y	4.62 (1.49-14.35)	1.53 (0.70-3.33)	0.12
3 y	2.86 (1.09-7.52)	1.38 (0.69-2.78)	0.20
5 y	2.22 (0.94-5.26)	1.18 (0.62-2.25)	0.27
	PSA < median† (n = 185)	PSA ≥ median† (n = 183)	
Tumor			
2 y	1.01 (0.27-3.69)	1.37 (0.70-2.68)	0.59
3 y	1.02 (0.34-3.03)	1.34 (0.72-2.51)	0.63
5 y	0.70 (0.27-1.82)	1.25 (0.69-2.27)	0.35
Nontumor			
2 y	1.21 (0.30-4.86)	2.41 (1.18-4.94)	0.25
3 y	0.84 (0.26-2.78)	2.18 (1.12-4.25)	0.13
5 y	0.78 (0.28-2.16)	1.91 (1.02-3.57)	0.14
	Stage II (n = 298)	Stage III or IV (n = 70)	
Tumor			
2 y	1.17 (0.49-2.78)	1.86 (0.80-4.31)	0.33
3 y	1.02 (0.48-2.18)	1.93 (0.88-4.28)	0.20
5 y	0.79 (0.41-1.54)	1.87 (0.87-4.00)	0.08
Nontumor			
2 y	2.22 (0.88-5.66)	2.53 (1.07-5.99)	0.62
3 y	1.66 (0.75-3.67)	2.14 (1.06-6.47)	0.53
5 y	1.32 (0.67-2.62)	1.96 (0.91-4.25)	0.47

NOTE: Clinical risk factors include PSA, tumor grade, and tumor stage except when stratified on one of these factors.
*P value for significant difference in HR between strata.
† Median PSA level was 5.1 ng/mL.

adduct levels in adjacent nontumor cells. To confirm that total DNA adduct burden in the prostate did not afford more information about BCR, we calculated a composite total score of PAH-DNA adducts based on a tumor volume–weighted average of PAH-DNA adducts in both tumor and nontumor cells, but found no associations with BCR (data not shown). Combining separate PAH-DNA adduct level measures in tumor and nontumor prostate cells into a composite score may dilute information in the adduct measure unique to each, particularly if the function of key genes in the adduct formation and repair pathways changes during malignant transformation (42, 43).

Nuclear accumulation of the p53 protein in prostate tumor cells has been associated with poor disease prognosis (44, 45). The diol epoxide metabolites of benzo(a)pyrene diol epoxide preferentially bind to the most frequently mutated guanine nucleotides within *p53* codons (46) and other forms of PAH also bind to *p53* mutational hotspots (47). Therefore, increased PAH-DNA adduct level in prostate leading to increased *p53* mutations may be a possible mechanism by which higher PAH-DNA adduct levels affect increased BCR in the short term. Another explanation for the association between higher PAH-DNA adduct levels and increased short-term BCR may lie in the metabolic environment of the premalignant cell. Cytochrome P450 phase I enzymes CYP1A1 and CYP1B1 activate PAH parent compounds. Allelic variants of CYP1A1 and CYP1B1, which may exhibit different catalytic capabilities toward PAH parent compounds (48), have been linked to aggressive prostate cancer (26). Furthermore, CYP1B1 is also overexpressed in prostate tumors due to hypomethylation (49).

A limitation of our study was that DNA adducts were measured cross-sectionally shortly after disease diagnosis. As such, the adduct level in our analysis was a snapshot of what could potentially be a rapidly changing cellular environment. That may explain in part why after 2 years the association between PAH-DNA adducts and BCR declined precipitously. Although our results are generalizable to prostate cancer patients that undergo prostatectomy as their primary form of treatment, we cannot necessarily infer that elevated PAH-DNA adduct levels affect disease progression the same way in prostate cancer patients that receive other forms of treatment such as hormone or radiation therapy. A missing aspect of the analyses in the present study that would be of interest in terms of prevention is the source of PAH exposure(s) that lead to high adduct levels and biological modifiers that influence adduct formation and prostate cancer risk, such as inherited capacities for high metabolism of PAH (8, 15) or poor DNA repair capacity (50, 51). Although these risk factors for PAH-DNA adducts have meaning in terms of understanding the underlying reasons for interindividual variation in PAH-DNA adduct levels, because they are antecedent factors in a causal pathway they provide no further understanding of the role of adducts in BCR (52), which was the central point of this study. This was evidenced by our rerunning of multivariate models including covariates for both PAH-DNA adduct levels and pack-years of cigarette smoking,

and our finding that inclusion of the latter did little to change the association of the former with the BCR outcome.

Interestingly, we found that the association of higher PAH-DNA adduct level with BCR was restricted to subsets of patients; in particular, those with advanced-stage disease, with PSA levels above the median at diagnosis, and those younger than 60 years old. Further stratified analysis also revealed that within the clinical patient subsets in which elevated DNA adduct levels were associated with BCR, African Americans were at greater risk for BCR. Both PSA and tumor stage are known risk factors for BCR and were strongly associated with BCR in our study population. Elevated PAH-DNA adducts may be a marker of a more advanced disease process involving activated phase I enzymes, which could have a greater effect in a disease progression process that has already exceeded a certain threshold as indicated by high PSA or advanced tumor stage. It is unclear why PAH-DNA adducts were a greater risk factor for BCR in men younger than 60 years old in our study. Neither age nor race was associated with BCR in our study population, but elevated DNA adduct levels had the strongest association with BCR in younger African-American cases. The combination of high adduct levels and younger age has been associated with higher risk of lung (53) and colorectal (54) cancer. Hu et al. (50) found lower nucleotide excision repair capacity was a stronger risk for prostate cancer in men younger than 60 years old and that the nucleotide excision repair capacity level was lower in younger cases, suggesting that deficient nucleotide excision repair capacity could contribute to early onset of prostate cancer. In a similar manner, high PAH-DNA adduct levels may better discriminate between aggressive and nonaggressive prostate cancer phenotypes in younger versus older cases. Few studies have examined racial differences in PAH-DNA adduct levels, but a study of smokers found that African American subjects had higher adduct levels in lymphocytes than Caucasian and Latino subjects after adjustment for gender, education, α-tocopherol and β-carotene levels, and *GSTM1* status (55).

In summary, we found that higher levels of PAH-DNA adducts in prostate were associated with a transient increased risk of BCR in men with prostate cancer treated with surgery. In patient subsets defined by high PSA, advanced tumor stage, and age less than 60 years old at diagnosis, higher adduct levels conferred an increased risk of BCR that diminished less with follow-up time and was greatest in African Americans. Higher adduct levels in nontumor cells compared with tumor cells tended to be more strongly associated BCR, which may be due to the cellular environment in nontumor cells being more reflective of an individual's innate ability to activate carcinogens and repair DNA damage. Our findings are novel and need to be replicated in independent populations. Future studies should also address whether DNA adducts in prostate cells at time of diagnosis are a harbinger of disease progression or simply a by-product of a cellular milieu already programmed for greater malignant potential.

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APPENDIX III

Abstracts Presented 2007-2008

Henry Ford Health System Research Symposium, Detroit, MI, Apr 13, 2007
A pilot study of telomere repeat binding factor 1 (TRF1) protein expression in prostate tumor and adjacent non-tumor cells of African-Americans and Caucasians.
C. Neslund-Dudas, S.P. Dudas, A.K. Meeker, X. Zhang, A.T. Savera, B.A. Rybicki

AACR - The Science of Cancer Health Disparities, Atlanta, GA, Nov 27-30, 2007
Coffee, beer, and wine consumption and PhIP-DNA adducts in black and white men with prostate cancer. **C. Neslund-Dudas**, D. Tang, C.H. Bock, A. Rundle, N. Nock, J. Beebe-Dimmer, B.A. Rybicki

AACR – Telomeres and Telomerase in Cancer, San Francisco, CA, Dec 6-9, 2007
A pilot study of telomere repeat binding factor 1 (TRF1) and telomere content in prostatectomy specimens of black and white men with prostate cancer. **C. Neslund-Dudas,** S.P. Dudas, A.K. Meeker, X. Zhang, A.T. Savera, R. Mikita, B.A. Rybicki.

Henry Ford Health System Research Symposium, Detroit, MI, Apr 13, 2007

A Pilot Study of Telomere Repeat Binding Factor 1 (TRF1) Protein Expression in Prostate Tumor and Adjacent Non-Tumor Cells of African-Americans and Caucasians

Background: African-American men develop prostatic intraepithelia neoplasia (PIN) and prostate cancer at younger ages than Caucasian men and telomeres have been shown to be progressively shorter in PIN and prostate tumor cells when compared to normal prostate cells. We hypothesized, therefore, that African-American men may in part develop prostate cancer at a younger age than Caucasian men because critical telomere length is reached earlier in life. Telomere repeat binding factor 1 (TRF1) is one of several proteins that form a complex which protects telomere ends. TRF1 binds directly to the t-loop structure formed by telomeres and negatively regulates telomere length by limiting the access of telomerase to the telomere. To our knowledge TRF1levels have not been previously reported in prostate tissue or by race. We present here our pilot work on TRF1 expression in tumor and adjacent non-tumor tissue of African-American and Caucasian prostate cancer cases.

Methods: Using paraffin embedded prostate tissue specimens and standard immunohistochemistry(antibody anti-TRFsc-1977, Santa Cruz Biotechnology), we assessed nuclear and cytoplasm TRF1 protein expression in a sample of African-American (n=24) and Caucasian (n=29) men with prostate cancer who participated in a larger case-control study. Semi-quantitative expression of the TRF1 protein in the cytoplasm was determined by grading signal intensity in combination with the percentage of positive staining cells. The degree of immunopositivity was obtained by multiplying the intensity and percentage of positive cells. Nuclear staining was quantified by count of cells (0, 1-5, >5), as very little nuclear staining was seen overall.

Results: Among all subjects TRF1 protein expression was lower in prostate tumor than adjacent non-tumor cells (nuclear staining of >5 cells: tumor 22.7% vs. non-tumor 41.5%, p-value=.01; cytoplasm mean immunopositivity ± standard deviation: tumor vs. non-tumor (0.53 ± 0.69 vs. 0.81 ± 0.01; p=.01). TRF1 expression was not associated with age (<60 years vs. ≥60 years), but TRF1 expression in non-tumor cytoplasm occurred less frequently in African Americans. Only half of African-American subjects (12/24) expressed TRF1 in at least 50% of prostate cells, compared to nearly 80% of Caucasians (23/29) (p=.04). History of hypertension was inversely associated with TRF1 expression in the cytoplasm of non-tumor prostate cells (percent of subjects with ≥50% of cells expressing TRF1: hypertensive 53.8% vs. normotensive 77.8%, p=.07; mean immunopositivity ± standard deviation for TRF1 expression: hypertensive vs. normotensive (0.64 ± 0.54 vs. 0.97 ± 0.54; p=.03)). In our sub-sample, African Americans and Caucasians had similar rates of hypertension; however, in the parent study and in general, African Americans are more likely to have hypertension than Caucasians.

Conclusions: Our pilot results indicate that African Americans and hypertensive individuals have a lower percentage of prostate cells expressing TRF1 protein in non-tumor cytoplasm suggesting possible biologic mechanisms for the observed racial differences in prostate cancer incidence. A larger study of race, hypertension, TRF1 expression and prostate cancer may be warranted.

AACR - The Science of Cancer Health Disparities, Atlanta, GA, Nov 27-30, 2007

Coffee, beer and wine consumption and PhIP-DNA adducts in black and white men with prostate cancer

Background: The predominant heterocyclic amine in cooked meats, 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP), has been shown to be carcinogenic in rat prostate and likely derives its carcinogenic potential through the formation of DNA adducts. Blacks have been shown to have higher levels of PhIP in their diet and urine. Coffee, beer and red wine, have been shown to affect PhIP adduct formation. Therefore, we tested whether consumption of these beverages was associated with PhIP-DNA adduct levels in prostate tissue.

Methods: The study included 105 black and 161 white men who underwent radical prostatectomy. Beverage and meat intake was assessed through food frequency questionnaires. PhIP-DNA adducts were measured using immunohistochemical methods. Data were analyzed using ANCOVA and stepwise linear regression.

Results: In tumor and adjacent non-tumor prostate tissue of all subjects, significantly lower mean PhIP-DNA adduct levels were found among beer (tumor: $p=0.01$, non-tumor: $p=0.03$) and red wine (tumor and non-tumor: $p< 0.01$) drinkers as compared to non-drinkers. Black men who consumed beer (tumor: $p= 0.01$, non-tumor: $p= 0.04$) or coffee (tumor and non-tumor: $p< 0.01$) had significantly lower mean adduct levels than blacks who did not consume these beverages. Among whites, red wine consumers (tumor: $p< 0.01$, non-tumor: $p= 0.03$) had significantly lower PhIP adducts. In total, 23% of black and only 5% of white men were non-drinkers of all three beverages. Interestingly, in stepwise regression analyses that included beverages, meat consumption was a significant predictor of PhIP adduct levels in whites but not blacks.

Conclusions: Although our previous work has shown that black and white men with prostate cancer do not differ quantitatively in PhIP-DNA adduct levels, the PhIP adducts in these two race/ethnic groups may result from different dietary exposures and therefore, may differ in mutagenic potential. More work is needed to identify dietary and genetic factors that impact PhIP-DNA adducts in diverse populations.

AACR – Telomeres and Telomerase in Cancer, San Francisco, CA, Dec 6-9, 2007

A pilot study of telomere repeat binding factor 1 (TRF1) and telomere content in prostatectomy specimens of black and white men with prostate cancer

Background: Telomeres have been shown to be progressively shorter in prostatic intraepithelial neoplasia (PIN) and malignant prostate cells when compared to normal prostate cells. Black men are known to develop PIN and prostate cancer at younger ages than white men, but to our knowledge there are no published reports of telomere content or telomeric proteins in prostate specimens by race. We, hypothesized, that black men may in part develop prostate cancer at a younger age than white men because critical telomere length is reached earlier in life and that components of the shelterin complex may play a role in racial disparities in age and incidence of prostate cancer onset. Telomere repeat binding factor 1 (TRF1) is one shelterin component that regulates telomere length by limiting access of telomerase to the telomere. We present here our initial pilot work on TRF1 expression and telomere content in black and white men with prostate cancer.

Methods: Using paraffin embedded prostate tissue specimens; we assessed TRF1 using standard immunohistochemistry (antibody anti-TRFsc-1977, Santa Cruz Biotechnology) and telomere content using telomere flourescence in situ hybridization (Teli-FISH). TRF1 protein expression was evaluated semi-quantitatively in both nuclear and cytoplasm of tumor and adjacent normal regions of 53 prostatectomy specimens. Expression of the TRF1 protein in the cytoplasm was determined by grading signal intensity in combination with the percentage of positive staining cells. The degree of immunopositivity was obtained by multiplying the intensity and percentage of positive cells. Nuclear staining was quantified by count of cells (0, 1-5, >5). Telomere content has been assessed in 26 of the 53 prostatectomy specimens thus far.

Results: Among all subjects TRF1 protein expression was lower in prostate tumor than adjacent non-tumor cells (nuclear staining of >5 cells: tumor 22.7% vs. non-tumor 41.5%, p-value=.01; cytoplasm mean immunopositivity ± standard deviation: tumor vs. non-tumor (0.53 ± 0.69 vs. 0.81 ± 0.01; p=.01). Only half of black subjects (12/24) expressed TRF1 in at least 50% of prostate cells, compared to nearly 80% of white cases (23/29) (p=.04). History of hypertension, a disease more common in black men, was inversely associated with TRF1 expression in the cytoplasm of non-tumor prostate cells (percent of subjects with ≥50% of cells expressing TRF1: hypertensive 53.8% vs. normotensive 77.8%, p=.07; mean immunopositivity ± standard deviation for TRF1 expression: hypertensive vs. normotensive (0.64 ± 0.54 vs. 0.97 ± 0.54; p=.03)).

Conclusions: Our pilot results indicate that black men have a lower percentage of prostate cells expressing TRF1 protein in non-tumor cytoplasm suggesting a possible biologic mechanism for the observed racial differences in prostate cancer incidence. A larger study of race, telomeres and prostate cancer may be warranted.

APPENDIX IV

CURRICULUM VITAE

CURRICULUM VITAE
Christine M. Neslund-Dudas

PERSONAL INFORMATION

Date of Birth: December 21, 1965

Place of Birth: Wayne, Michigan

Home Address: 32470 Norfolk
Livonia, MI 48152

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RESEARCH INTERESTS

Exploring the social and biological factors that work in concert to produce race disparities in health. Areas of interest include: the influence of comorbidity on cancer development, diagnosis and progression, prostate cancer, lung cancer, and end-of-life care.

EDUCATION

Wayne State University	BS	1988	Biology
Wayne State University	MAT	1994	Secondary Science Education
Wayne State University	Non-degree coursework	1996-1999	Community Medicine (Biostat/Epidemiology)
Wayne State University	PhD-all course work completed	Anticipated Fall 2008	Medical Sociology

PROFESSIONAL EXPERIENCE

1988-1994: Infection Control Technician, Hospital Epidemiology, Henry Ford Health System, Detroit

1994-1997: Project Coordinator, Cntr for Health System Studies, Henry Ford Health System, Detroit

1997-1999: Sr. Project Coordinator, Cntr for Health System Studies, Henry Ford Health System, Detroit

1999-2001: Epidemiologist I, Josephine Ford Cancer Center, Henry Ford Health System, Detroit

2001-2006: Epidemiologist II, Biostatistics Research Epidemiology/ Cancer Epidemiology Prevention and Control, Henry Ford Health System, Detroit

2007-present: Epidemiologist III, Biostatistics Research Epidemiology/ Cancer Epidemiology Prevention and Control, Henry Ford Health System, Detroit

HONORS

WSU Graduate Professional Scholarship (2003-2004, 2005-2006, 2006-2007)

Henry Ford Health System Research Symposium, Abstract Selected for Presentation (2005)

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Tang D, Lui JJ, Rundle A, **Neslund-Dudas C**, Bock CH, Nock NL, Yang JJ, Rybicki BA. Grilled Meat Consumption and PhIP-DNA Adducts in Prostate Carcinogenesis. *Cancer Epidemiol Biomarkers Prev*, 2007; 16(4):803-8.

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Rybicki BA, Neslund-Dudas C, Bock CH, Rundle A, Savera AT, Yang JJ, Nock NL, Tang D. Polycyclic Aromatic Hydrocarbon—DNA Adducts in Prostate and Biochemical Recurrence After Prostatectomy. *Clin Cancer Res*, 2008;14(3):750-7.

ABSTRACTS AND PRESENTATIONS

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Rybicki B, **Neslund-Dudas C**, Sankey S, Thomas D, Monaghan K, Amend K. Interaction between Dietary Fat Intake and the Androgen Receptor CAG Repeat Polymorphism in Prostate Cancer. *Society for Epidemiological Research***.** Salt Lake City, Utah. June 2004.

Neslund-Dudas C, Schultz L, Monaghan K, Bock C, Rybicki B. 3 Beta-Hydroxysteroid Dehydrogenase Type II Polymorphism, Age And Race In Prostate Cancer. *Gerontology Society of America*. Orlando, FL. Nov 18-21, 2005

Herrinton LJ, Hornbrook M, Rolnick SJ, **Neslund-Dudas C**, Bachman D, Jackson J, Coughlin SS. Ovarian Cancer Palliative Care Study: Preliminary Findings. *CDC.* Atlanta, GA. June 2005

Rolnick S, Jackson J, Nelson W, Butani A, Herrinton LJ, Hornbrook M, Bachman D, **Neslund-Dudas C**, Coughlin S. Pain Management in the Last Six Months of Life for Women Who Died of Ovarian Cancer. *American Society of Preventive Oncology (30th Annual Meeting).* Bethesda, Maryland. February 26-28, 2006

ABSTRACTS AND PRESENTATIONS (CONTINUED)

Rybicki B**, Neslund-Dudas C,** Savera A, Rundle A, Nock N, Bock C, Yang JJ, Tang D. Polycylic Aromatic Hydrocarbon-DNA adduct Levels are e Predictor of Biochemical Recurrence in Prostate Cancer Patients undergoing prostatectomy. *American Assoc. for Cancer Research*. Charleston, South Carolina. January 11-15, 2006.

Neslund-Dudas C, Krajenta R, Jacobsen G, Hornbrook MC, Bachman DJ, Rolnick SJ, Jackson J, Coughlin SS, Herrinton LJ. Comorbidity and Symptoms in Women Dying of Ovarian Cancer. *HMO Research Network Conference (12th Annual).* Boston, MA. May 1-3, 2006.

Neslund-Dudas C, Tang D, Savera A, Rundle A, Bock CH, Rybicki BA. Race-Specific Determinants of PhIP-DNA Adducts in Prostate Cancer. *HFHS Research Symposium (3rd Annual).* Detroit, MI. April 21, 2006.

Krajenta R, Jacobsen G, Hornbrook MC, Bachman DJ, Rolnick SJ, Coughlin SS, Herrinton LJ, **Neslund-Dudas C.** Comorbidity and Symptoms in Women Dying of Ovarian Cancer. *HFHS Research Symposium (3rd Annual)*. Detroit, MI. April 21, 2006.

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Jackson JM, Rolnick SJ, Coughlin SS, **Neslund-Dudas C**, Hornbrook MC, Darbinian J, Bachman DJ, Herrinton LJ. Social Support Among Women Who Died of Ovarian Cancer. *HMO Research Network Conference (12th Annual).* Boston, MA. May 1-3, 2006.

Neslund-Dudas C, Dudas SP, Meeker AK, Zhang X, Savera AT, Rybicki BA.
A Pilot Study of Telomere Repeat Binding Factor 1 (TRF1) Protein Expression in Prostate Tumor and Adjacent Non-tumor Cells of African-Americans and Caucasians. *AACR Translational Research at the Aging and Cancer Interface*. San Diego, CA. Feb 20-23, 2007.

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A pilot study of telomere repeat binding factor 1 (TRF1) protein expression
in prostate tumor and adjacent non-tumor cells of African-Americans and Caucasians.
Henry Ford Health System Research Symposium, Detroit, MI, Apr 13, 2007

Neslund-Dudas C, Tang D, Bock CH, Rundle A, Nock N, Beebe-Dimmer J, Rybicki BA.
Coffee, beer, and wine consumption and PhIP-DNA adducts in black and white men with prostate cancer. *AACR - The Science of Cancer Health Disparities, Atlanta, GA, Nov 27-30, 2007*

Neslund-Dudas C, Dudas SP, Meeker AK, Zhang X, Savera AT, Mikita R, Rybicki BA.
A pilot study of telomere repeat binding factor 1 (TRF1) and telomere content in prostatectomy specimens of black and white men with prostate cancer. *AACR – Telomeres and Telomerase in Cancer, San Francisco, CA, Dec 6-9, 2007*

CURRENT FUNDING

Department of Defense –Pre-Doctoral Training Grant Neslund-Dudas(PI) 3/15/ 2007-3/14/2009
W81XWH-07-1-0252
Role: PI
Residential Segregation, Housing Status, and Prostate Cancer in African-American and White Men
This training grant is assessing the relationship between individual and area measures of housing and prostate cancer outcomes (age at diagnosis, aggressiveness, recurrence) in African-American and White Men. The study is using previously enrolled and well defined prostate cancer cases, diagnosed at a large health system serving the Metropolitan Detroit Area.

NIH/NIEHS Rybicki (PI) 6/01/07-2/28/12
R01 ES011126-06 A2
Role: Project Manager
Nested Case Control Study of Prostate Carcinogenesis
The primary aims of this study are 1) to determine whether biomarkers of DNA damage (PAH-andPhIP-DNA adducts) are predictive of prostate cancer (CaP) development, 2) to determine in a multivariable model how known markers of progression to CaP (i.e., DNA methylation affect the association between PAH-and PhIP-DNA adducts and prostate cancer and 3) to determine whether DNA adducts in the benign prostate are associated with the level of expression of p53 and p21waf/cip1 tumor suppressor genes in subsequent prostate tumors.

R01 CA088164-07A2 Witte (PI) 12/01/06-11/30/11
Role: Project Manager
Genetic Epidemiology of Prostate Cancer Aggressiveness
The purpose of this application is to evaluate the impact of candidate genes involved with the innate immunity and inflammation pathway and nonsteroidal anti-inflammatory drugs (NSAIDs) on prostate cancer development and progression.

OTHER RECENT SUPPORT

NIH/NIEHS 5 R01 ES11126-03 Rybicki (PI) 9/30/00 – 7/31/05
Role: Project Manager
Gene-Environment Interaction in Prostate Cancer
The objective of this project is to identify combinations of genetic and environmental risk factors that increase a man's risk for prostate cancer above what would be expected if the risk factors acted independent of each other. Hypotheses are focused on occupational and dietary risk factors and the genes that may modify them based on our current understanding of prostate carcinogenesis.

CDC Task Order 200-95-0953-953-047 10/1/2000-9/30/2002
Role: HFHS Site PI
Evaluation of End-of-Life Care for Prostate Cancer in the Managed Care Environment
This retrospective study enrolled nearly 500 subjects who had died of prostate cancer between 1993 and 2000. Medical records including inpatient, outpatient and hospice data were abstracted. Participating sites included Group Health Cooperative (Seattle) and Henry Ford Health System (Detroit).

NCI 5U01CA093332-04 Weeks (PI) 9/18/01-8/31/06

Role: HFHS Project Manager (2001-2004)

Lung/Colon Cancer Outcomes--Cancer Research Network

This RFA cooperative agreement (RFA-CA-01-013) supports a new collaborative research consortium to conduct Cancer Care Outcomes Research and Surveillance (CanCORS), NCI's first major step to support the development of a system for obtaining details about cancer care beyond the initial diagnosis and limited treatment data that are now routinely collected in high quality population-based cancer registries. This research will help build the information base needed for measuring and improving the quality of cancer care in the US. CanCORS purpose will be to collaboratively collect and analyze process-outcome relationships in patients newly diagnosed with lung or colorectal cancer.

CDC Task Order 2002-Q-00654 10/1/2002-1/31/2006

Role: HFHS Site PI

Evaluation of Hospice Referral and Palliative Care for Ovarian Cancer in the Managed Care Environment

This retrospective study is evaluating end-of-life care for women who died of ovarian cancer. Participating sites include Kaiser Permanente Northern California, Kaiser Permanente Northwest, and Henry Ford Health System. Data has been collected for more than 400 women and is now being analyzed

NIH/NIEHS 3 R01 ES011126-02S1 Rybicki (PI) 8/1/02 – 7/31/05

Role: Project Manager

Determinants of PAH-DNA Adducts in Prostate Cancer

The main objective of this project is to identify combinations of genetic and environmental risk factors that determine levels of polycyclic aromatic hydrocarbon (PAH) DNA adducts in prostate cancer tissue.

NCI U19 CA 79689 Wagner (PI), CC Johnson (HFHS Site-PI) 3/1/03 – 2/28/07

Role: HFHS Project Manager Infrastructure

Increasing Effectiveness of Cancer Control Interventions: HMO Cancer Research Network:

The goal of this program is to determine and improve the effectiveness of cancer control interventions that span the natural history of major cancers among diverse populations and health systems.

Karmanos Cancer Institute –Strategic Research Initiative Rybicki (PI) 1/1/2006-12/31/2006

Role: Lead Writer / Analyst

Race Disparities In Prostate Cancer: Do Telomeres Play A Role?

Research on telomeres as biomarkers of prostate cancer and as biomarkers of socioeconomic impact on health are converging to highlight the potential importance of telomere assessment in health disparities research in PCa. The objectives of this pilot study are to demonstrate our ability to quantify and assess telomere content using paraffin embedded tumor and tumor-adjacent normal tissue of prostate cancer cases and begin to determine if there are associations between race and telomere content in prostate tissue. Also, we will demonstrate our ability to assess telomere repeat binding factor-one (TRF1) levels in tumor and tumor adjacent-normal prostate tissue and begin to determine if there is an association between TRF1, race and telomere length. (TRF1 is a negative regulator of telomere length.)

Robert Wood Johnson Foundation Nerenz (PI) 4/1/2007- 3/31/2008

Role: Data Set Support

Reanalyzing data sets using path analysis and structured equation modeling to assess racial and ethnic health care disparity